INTERACTIONS OF CONTAMINANTS, STRESS AND PHYSIOLOGICAL CONSEQUENCES IN MALE LESSER SCAUP (*AYTHYA AFFINIS)* FROM THE NORTHERN BOREAL FOREST

A thesis submitted to the College of
Graduate Studies and Research in partial
fulfillment of the requirements for the
degree of Master of Science in the
Toxicology Graduate Program at the
University of Saskatchewan, Saskatoon

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ABSTRACT

In the mid-1980's until the late 1990's, Lesser Scaup (*Aythya affinis*) populations in the boreal forest declined and have remained at historical low levels since that time. This has resulted in a population well below conservation goals. Potential causes for this population decline include a reduction in productivity, which could be related to changes in boreal forest habitat, nutritional condition during reproduction, or due to contaminants acquired during migration or wintering. Though several studies have assessed contaminant levels in Lesser Scaup on wintering, staging and migration routes, relatively little data exist from northern boreal forest areas, one of the core breeding habitats of the Lesser Scaup population and where population declines appear to be most severe. To this end, male Lesser Scaup were trapped from sites in the northern boreal forest in 2004 and 2005 to assess trace element contaminant levels. Previous research has shown that trace elements including cadmium and selenium can influence hormonal status in waterfowl. Specifically, a positive relationship between cadmium and corticosterone and a negative correlation between liver selenium and corticosterone have been observed. The purpose of this study was to test the hypothesis that trace element contaminants can influence hormonal status and related physiological functions in male Lesser Scaup, and that interactions between contaminants, physiological variables such as body condition and social status can modify expression of toxic effects. Blood samples were collected from trapped males to assess stress related changes in blood chemistry (corticosterone, testosterone, glucose and thyroxine) and males were then collected for contaminant analysis and assessment of reproductive physiology. The geometric mean levels of kidney cadmium, liver selenium and liver mercury were 9 μg/g, 4.33 μg/g, 1.31 μg/g dry weight respectively. Several variables and interactions including pair status, cadmium, selenium, mercury, body condition and body size influenced corticosterone levels. In male Scaup with high cadmium levels, corticosterone was negatively related to liver selenium in birds with good body condition (R^2=0.701,

n=9, P=0.005) but not in birds with poor body condition (R^2=0.033, n=10, P=0.61). Likewise, in birds with high cadmium, a negative association was found between liver mercury and corticosterone in structurally smaller males (R^2=0.491, n=10, P=0.024), whereas no such relationship was found in larger males (R^2=0.307, n=9, P=0.12). In birds with low cadmium and low mercury, selenium and corticosterone were negatively correlated (R^2=0.568, n=10, P=0.012) while no association was found in males with high mercury (R^2=0.325, n=10, P=0.085). Unpaired birds had higher corticosterone than in ducks with low cadmium ($F_{1,17}$=6.70, P=0.023), while there was no difference between groups in ducks with high cadmium. Glucose levels were not influenced by contaminants or other variables in this study (R^2=0.551, $F_{21,17}$=0.99, P=0.51). Thyroxine levels were positively correlated to mercury levels in paired birds (R^2=0.485, n=19, P<0.001) but were not related in unpaired birds (R^2=0.063, n=20, P=0.28). Thyroxine levels also showed a relationship with date of capture ($F_{1,37}$=6.77, n=39, P=0.014). Pair status was influenced by body condition and body size (χ^2 =9.997, df=2, P=0.007) with larger birds being in better condition and larger, while hormone levels and testicular morphology did not appear to influence pair status. Mass of testes ($F_{9,27}$=0.45, P=0.90) and testosterone concentrations ($F_{10,28}$=0.31, P=0.10) were not influenced by contaminant levels, body condition or body size. Seminiferous tubule diameter was positively related to testes mass (R^2=0.397, n=39, P<0.0001) and negatively related to liver selenium levels (R^2=0.123, n=39, P=0.009). To clarify the influence of selenium on corticosterone, captive male Lesser Scaup were fed diets dosed with selenomethionine. Dose groups accumulated different levels of selenium (control group=0.19 ng/ml, 7.5 ppm group=0.74 ng/ml and 15 ppm=1.01 ng/ml) ($F_{2,12}$=155.12; P<0.001) but this appeared to have no effect on corticosterone levels (Wilks' Lambda=0.659, $F_{4,10}$=1.29, P=0.34) or behavioral patterns (Wilks' Lambda=0.659, $F_{4,10}$=1.294, P=0.34) in captive birds. Results from the field portion of this study support the hypothesis that trace element levels can influence hormonal status in wild Lesser Scaup and that interactions of

contaminants and physiological variables can modify expression of toxic effects. Studies such as this one display the complex nature of biological systems and emphasize the importance of considering interactions between different contaminants and other variables to clearly assess their influences on physiology.

ACKNOWLEDGMENTS

I would like to thank my supervisor Dr. Karen Machin for her support and guidance throughout this project and preparation of this thesis. I would also like to thank my committee members, Dr. Bob Clark (CWS) and Dr. Barry Blakley for their advice and generously donated time. Also thank you to Dr. Bob Brua who provided much needed assistance and guidance while working through statistical analysis of data.

Funding for this project was graciously provided by Environment Canada Science horizons Youth Scholarship program and supervisors funding from the National Sciences and Engineering Research Council.

The extensive field and laboratory work required for this project would not have been possible without the help of many individuals including Jane Harms, Jamille Heer, Jim Hines and the folks at CWS Yellowknife, Heather James, Steve Leach and Victoria May. I also appreciate the always sage advice from various faculty members at the Western College of Veterinary Medicine and the Toxicology Centre including Dave Janz, Lynn Weber and Mark Wickstrom.

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ACTH . . . . . adrenocorticotropin hormone

Cd . . . . . cadmium

CORT . . . . . corticosterone

dw . . . . . dry weight

FSH . . . . . follicle stimulating hormone

GLM . . . . . general linear model

Hg . . . . . mercury

HPA . . . . . hypothalamus-pituitary-adrenal axis

LH . . . . . luteinizing hormone

ppm . . . . . parts per million

RIA . . . . . radioimmunoassay

Se . . . . . selenium

T_3 . . . . . triiodothyronine

T_4 . . . . . thyroxine

ww . . . . . wet weight

1.0 General Introduction

1.1 Background

Wildlife populations have commonly been used as indicators of ecosystem health. This includes North American waterfowl populations, which can be influenced by condition of aquatic systems and wetlands. As human activity, developments and populations continue to increase, declines in specific wildlife populations may provide evidence of damage caused to habitats by these changes. This damage to the environment can include changes in food resources or availability, loss of important breeding habitats or contamination of the ecosystem. The result may impact on individual physiology or cause a decline in reproductive success which can ultimately lead to a decline of a population.

Contaminant levels in aquatic environments and within tissues of waterfowl species may be an important indicator of compromised ecosystem health. Quantifying these contaminant levels may not be an accurate assessment of actual impacts of elevated levels on health of individuals or populations. Comparison of contaminant levels to physiological variables among individuals may clarify impacts

and allow for improved prediction of effects on the population. Though considerable research exists regarding mechanism's of action and threshold levels for toxic effects of specific contaminants, the extrapolation of results to wild populations is often hampered by a number of factors. Species sensitivity, contaminant interactions and habitat characteristics can all influence or modify the expression of toxic effects. Therefore research, should be directed towards assessing the influence of contaminants on the physiology of specific species which have shown altered population structure and in the habitats considered most important to their life history.

1.2 Lesser Scaup (*Aythya affinis*)

1.2.1 Natural History

Feeding Habits and Diet

Lesser Scaup are diving ducks, meaning they forage primarily by diving for prey. These ducks will feed on free swimming prey in the water column, on prey adhered to the surface of substrate, and on prey within the sediment by inserting bill into the substrate (Austin et al., 1998). Diet can vary with season, breeding cycle and habitat. Dominant food sources for breeding adults in the Northwest Territories are generally soft-bodied invertebrates such as midges (Diptera: Chironomidae) and leeches (Hirudinea) (Bartonek and Murdy, 1970). Juvenile and duckling diets in the Northwest Territories appear to be dominated by amphipods (Amphipoda), mollusks, midges and other invertebrates, though prey choice and proportions appear to vary with duckling age (Bartonek and Murdy, 1970). During

migration through the Mississippi River system, diet can vary considerably, sometimes dominated by high protein food sources such as invertebrates, while in other years diet can be dominated by plant material despite the availability of invertebrate prey (Smith and Eichholz, 2006). During spring and fall migration through Minnesota, a majority of the diet historically was made up of amphipods (Amphipoda) and snails (Afton and Hier, 1991), though recent studies indicate a shift to other prey such as chironomids (Anteau and Afton, 2006). Prior to the Zebra Mussel (*Dreissena polymorpha*) invasion, the diet of Lesser Scaup on the lower Great Lakes was dominated by native non-filter feeding gastropods (Ross et al., 2005). Since the invasion of non-native Zebra Mussels and closely related Quagga Mussels (*Dreissena bugensis*),the diet of Lesser Scaup can be comprised of up to 98% of these filter feeders (Custer and Custer, 2000). This percentage may vary between spring and fall migration with Lesser Scaup typically consuming more Zebra Mussels in the spring than in the fall (Petrie, 2004). This shift of diet to filter feeding prey could contribute to an increased risk of contaminant exposure for Lesser Scaup wintering or staging on the southern Great Lakes (Ross et al., 2005).

Range and Migratory routes

Lesser Scaup are abundant, with wintering ranges including much of coastal and southern United States and extending into Central America and the Gulf of Mexico (Austin et al., 1998). Breeding ranges include much of mid-continental Canada though the most important nesting habitats are located in the boreal forests and parklands extending through the Canadian prairies and into the Northwest

Territories and Alaska (Austin et al., 1998). Several migratory routes are thought to be used by Canadian and northern breeding Lesser Scaup. These include: the Mississippi Flyway, which extends from the coast of the Gulf of Mexico along the Mississippi River into the southern great lakes; the Central Flyway, including the prairie pothole region and boreal forest edges; the Pacific Flyway, thought to involve movement directly over the Pacific ocean and into Alaska; and the Atlantic Flyway involving movement from the Atlantic coast through the Great Lakes (Bellrose, 1980). Some Lesser Scaup breeding in the Northwest Territories are thought to migrate to the Gulf or Atlantic coasts (Austin et al., 1998) and a significant proportion likely use the Mississippi and Atlantic Flyways during migration. Satellite tracking of Lesser Scaup during spring migration has shown that birds marked on the lower Great Lakes were widely dispersed through the boreal forest at the completion of migration, including sites in northern Ontario, Manitoba, the Northwest Territories, Yukon and Alaska (Badzinski and Petrie, 2006). Important staging areas for migrants utilizing the Mississippi and Atlantic flyways include the southern Great Lakes and surrounding wetlands and rivers, Lake St. Clair, Delta marsh in Manitoba and other lakes and rivers across Manitoba and Minnesota (Bookhout et al., 1989; Austin et al., 1998). Numbers of Lesser Scaup and other diving duck species using the southern Great Lakes as wintering and staging areas during migration appear to have dramatically increased since 1990, likely in response to proliferation of the invasive Zebra Mussel which provides an abundant and easily accessible food source (Wormington and Leach,

1992; Custer and Custer, 1996; Custer and Custer, 2000; Petrie and Schummer, 2002).

Breeding and Pairing Behavior

Lesser Scaup are seasonally monogamous (Anderson and Titman, 1992) and are considered a late-pairing species, with most pairing occurring during late-spring migration (Austin et al., 1998). These ducks typically arrive on breeding grounds in the Northwest Territories in mid-May though dispersal from migratory rafts to nesting wetlands may be delayed by a late spring thaw. Pairs can be identified by mutual preening behavior with mate, vocalizations and displays such as the Head-Throw Display (Austin et al., 1998). Lesser Scaup are considered an upland-nesting species, though in the Northwest Territories nests are typically built within dense, bunched sedge within 1 meter of water (Austin et al., 1998). Large wetlands (>50m^2) appear to be the preferred nesting habitat for pairs breeding in the Northwest Territories (Toft et al., 1982) with nest initiation typically occurring in early June (Austin et al., 1998). Lesser Scaup utilize a female-only incubation strategy with hens leaving the nest to forage for approximately 3.5 hours per day (Afton and Paulus, 1992). Males leave the nesting site during mid- to late-incubation and form large flocks with other males and non-breeding females on molting lakes (Bellrose, 1980; Austin et al., 1998). The preferred brood-rearing habitat in the northern boreal forest appears to be on large, deep wetlands with yellow waterlilies (*Nuphar latifolia*) and abundant amphipods (Fast et al., 2004).

1.2.2 Population Status

The North American Waterfowl Management Plan (NAWMP, 2004) is an international conservation initiative involving Canada, the United States and Mexico, with the goal of sustaining and restoring North America's waterfowl populations. To this end, population objectives, derived from average breeding populations during the 1970's, have been set for North America's waterfowl species. In the mid-1980's until the late 1990's, Lesser Scaup populations in the boreal forest declined and have remained at historical low levels since that time (NAWMP, 2004; Ducks Unlimited, 2005). This has resulted in a population well below conservation goals. Though difficulties with field identification of Greater (*Aythya marila*) and Lesser Scaup prevent separate counts during surveys, it is estimated that Lesser Scaup comprise approximately 89% of the total Scaup population (Austin et al., 2000). Accompanying the population decline have been two additional shifts in Lesser Scaup demographics: a decrease in the proportion of young Lesser Scaup which may indicate a decrease in reproductive success and an increase in the proportion of male to female Lesser Scaup, possibly indicating low adult female survival.

The most dramatic declines have occurred in the important boreal forest breeding habitats and could indicate the influence of changing boreal habitat on Scaup population dynamics (Austin et al., 2000). This theory appears to be supported by a parallel decline of scoter populations in the Northwest Territories since the 1970's (NAWMP, 2004). Though the causes of Scaup population declines are not known, several theories have been proposed. Lower female

survival, changes in food resources and breeding habitats, and contaminants are currently thought to be primary factors contributing to declining populations (Austin et al., 2000). Several courses of action have been recommended by leading waterfowl researchers in an attempt to clarify mechanisms of declining Scaup numbers including an assessment of contaminant effects on reproduction and survival (Austin et al., 2000).

1.2.3 Contaminants in Lesser Scaup

Several studies have assessed levels of contaminants in Lesser Scaup across North America, though a majority of research has focused on Mississippi flyway and Great Lakes regions. Trace element analysis has been conducted on Lesser Scaup across North America. Levels of most essential and non-essential trace elements were considered to be below toxic levels for Lesser Scaup collected from the Mississippi flyway. Mean liver cadmium concentrations however, averaged 2-3 μg/g dry weight in Lesser Scaup from sites on the southern Great Lakes, with 44% (Custer and Custer, 2000) and 34% (Custer et al., 2000) of birds having liver cadmium concentrations above levels considered background (3 μg/g dry weight) (Di Giulio and Scanlon, 1984b; Scheuhammer, 1987). Cadmium concentrations from other sites on the Mississippi flyway were lower than those reported for industrial portions of the Great Lakes with mean liver concentrations of 0.62 μg/g dry weight (Custer et al., 2003). Lesser Scaup from the Chesapeake Bay region on the Atlantic coast had mean liver cadmium levels of 1.25 μg/g dry weight (Di Giulio and Scanlon, 1984a). Kidney cadmium levels in Scaup collected from San

Francisco Bay, California showed some site and seasonal variation with mean tissue levels ranging from 0.61 to 33.9 μg/g dry weight with a maximum recorded level of 85 μg/g dry weight (Hothem et al., 1998). Takekawa et al (2002) reported kidney cadmium levels for Lesser Scaup from coastal California ranging from 0.58 to 7.08 μg/g dry weight with a maximum value of 18 μg/g dry weight.

Liver mercury concentrations from the Great Lakes and Mississippi Flyway have typically been low, with mean levels ranging from 0.96 (Custer et al., 2003) to 1.12 μg/g dry weight (Custer et al., 2000). Liver mercury levels in Scaup from sites on San Francisco Bay ranged from 2.37 to 10.4 μg/g dry weight (Hothem et al., 1998) and from coastal California ranging from 2.3 to 7.58 μg/g dry weight (Takekawa et al., 2002).

Selenium has been detected at levels considered elevated in Lesser Scaup throughout the Great Lakes region. Normal liver selenium levels for birds living in freshwater habitats range from 4 to 10 μg/g dry weight (Ohlendorf, 1989). On southern Lake Michigan, 88% of Scaup collected had selenium levels above normal levels (Custer et al., 2000). Mean liver concentrations of selenium in Lesser Scaup from other locations on the southern Great Lakes have been recorded at 21.7 μg/g dry weight (Custer and Custer, 2000) and 24.8 μg/g dry weight (Petrie, 2004), though selenium body burdens for Lesser Scaup captured in the fall dropped to a mean levels of 7 μg/g dry weight. Liver selenium levels in Lesser Scaup collected between November 1999 and May 2000 from sites throughout the Mississippi flyway ranged from 4.23 to 10.7 μg/g dry weight (Custer et al., 2003). Selenium

concentrations in Scaup from the San Francisco Bay region ranged from 3.55 to 32.7 μg/g dry weight (Hothem et al., 1998) and from 3.5 to 11.93 μg/g dry weight from other sites along coastal California (Takekawa et al., 2002).

Comparatively little toxicological data exist from Lesser Scaup breeding in areas in the northern boreal forest. Fox et al. (2005) assessed contaminant levels in 10 female Lesser Scaup collected from central Saskatchewan, Northwest Territories, and Alaska. Kidney cadmium concentrations in collected females ranged from 1.49 to 6.21 μg/g dry weight and liver mercury concentrations ranging from 0.39 to 1.77 μg/g dry weight. Liver selenium concentrations ranged from 2.60 to 5.27 μg/g dry weight. Another study measured mercury and selenium levels in other waterfowl species collected between 1988 and 1995 from sites in northern Canada (Braune and Malone, 2006). Some individuals from several species, including Green-winged Teal (*Anas crecca*), Bufflehead (*Bucephala albeola*), King Eider (*Somateria spectabilis*), Barrow's Goldeneye (*Bucephala islandica*), Common Goldeneye (*Bucephala clangula*), Surf Scoter (*Melanitta perspicillata*) and White-winged Scoter (*Melanitta fusca*), had liver selenium above 3 μg/g wet weight, indicating the potential for reproductive impairment and teratogenesis (Heinz, 1996). The highest concentrations were observed were in King Eider (2.6 to 20 μg/g wet weight), White-winged Scoter (1.2 to 20 μg/g wet weight) and Surf Scoters (3.7 to 13 μg/g wet weight). Mercury concentrations in liver were below the threshold for major toxic effects of 5 μg/g wet weight (Zillioux et al., 1993), with levels ranging from 0.016 to 3.8 μg/g wet weight.

1.3 Sources and Effects of Trace Element and Contaminants

1.3.1 Selenium

Selenium is a semi-metallic element which occurs naturally in soil, plants and rocks in four oxidation states: elemental selenium (Se^0), selenide (Se^{2-}), selenite (Se^{4+}) and selenate (Se^{6+}) with the latter two being the most bioavailable of the inorganic forms (Goyer and Clarkson, 2001). Selenate and to a lesser extent selenite, which is less soluble and mobile than selenate, are leached from soils becoming available for uptake by plants (Ohlendorf, 1989). Plants can convert these inorganic forms to organic forms, including selenocysteine and selenomethionine (Spallholz and Hoffman, 2002). Though the dominant route of exposure for most animals is through diet, aquatic organisms can absorb selenium directly from the surrounding water (Ohlendorf, 1989). Animals absorb dietary selenite, selenate and some organic forms of selenium through the small intestine where it is distributed to a variety of organs, with highest accumulation in liver and kidney though significant levels remain in blood and other tissues (Goyer and Clarkson, 2001). Primary routes of excretion for selenium are in urine and feces, though sweat and respiration may also secrete small amounts (Goyer and Clarkson, 2001).

Selenium is an essential element for animal nutrition and deficiency has been shown to be responsible for a number physiological disorders such as dietary liver necrosis in rats (Goyer and Clarkson, 2001) and pancreatic atrophy in chicks (Ohlendorf, 1989). Dietary requirements for most animals typically range from

0.05 to 0.3 mg/kg/day (Ohlendorf, 1989). Selenium is an important component of several proteins including the glutathione peroxidase enzymes (Spallholz and Hoffman, 2002) which serve a protective function against oxidants and free radicals, and the iodothyronine deiodinase enzyme system which catalyzes the conversion of thyroxine (T_4) to triiodothyronine (T_3)(Goyer and Clarkson, 2001).

Anthropogenic activities can account for significant contributions of selenium into the environment. Fly ash from coal power plants, mining of metals, and runoff from agricultural activities can contribute to selenium contamination of soil and aquatic systems (Hoffman, 2002). Once in the environment, selenium can enter the foodweb resulting in trophic transfer of elevated levels of selenium from lower organisms such as zooplankton, through insects and bivalves, to upper level consumers (Ohlendorf, 1989). Though bioconcentration of waterborne selenium by algae occurs in some systems, with an average bioconcentration factor of approximately 1400, subsequent transfers up the food chain to macroinvertebrates averaged only 1.9 fold increase in selenium concentration (Fan et al., 2002). Other studies have shown that selenium concentrations in fish and invertebrates range from 2 to 6 times higher than those in producers such as algae (Ohlendorf, 1989). It is thought that proteinaceous forms of selenium including selenomethionine are the dominant forms of selenium transferred through the food chain (Fan et al., 2002). Trophic transfer of selenium is thought to be responsible for elevated concentrations of selenium observed in Lesser Scaup from the Great Lakes region. Zebra Mussels, which were introduced into the Great Lakes system in 1986 and

have since become the preferred food source for wintering and staging diving ducks on the southern Great Lakes, accumulate a variety of contaminants in their tissue during filter feeding (Berney et al., 2003; Petrie, 2004). Zebra Mussels from Lake Erie had spring body burdens of selenium averaging 10.8 μg/g dry weight, well above the suggested 3 μg/g toxic threshold for consumption of aquatic organisms by wildlife (Petrie, 2004).

The impacts of selenium toxicity in aquatic birds have been well documented. Based on field and laboratory studies, a tissue level of 10 μg/g wet weight in liver, has been suggested as the threshold for major toxic effects to adult and young birds, while 3 μg/g wet weight in the liver of females may be associated with reproductive impairment (Heinz, 1996). Several captive studies have assessed effects of selenomethionine on reproduction in Mallards (*Anas platyrhynchos*). In a feeding trial, Mallards were dosed with 1, 5, 10 or 25 μg/g sodium selenite or 10 μg/g D,L-selenomethionine (Heinz et al., 1987). In this study, 10 μg/g selenomethionine and 25 μg/g sodium selenite caused a significant reduction in the number of eggs that hatched and 10 μg/g of selenomethionine was more teratogenic than sodium selenite. These reproductive effects were also observed in another study, in which Mallards were fed diets supplemented with D,L-selenomethionine or D,L-selenocystine (Heinz et al., 1989). Selenocystine supplemented diet had no impact on reproductive success in Mallards. Selenomethionine at 8 and 16 μg/g was teratogenic (affecting eyes, bills, legs and feet) in 6.8 and 67.9% of chicks respectively in unhatched eggs and also reduced the number of chicks that survived

until 6 days post hatch. Selenomethionine tended to accumulate in eggs to a greater degree than selenocystine. When Mallard ducklings were fed diets dosed with sodium selenite and D,L-selenomethionine, there was decreased growth at 20 μg/g and reduced survival at 40 μg/g with both forms (Heinz et al., 1988). In a similar study there was decreased growth in ducklings fed diets supplemented with 15 μg/g selenomethionine (Hoffman et al., 1991).

Reproductive toxicity of selenium has been observed in field studies. Kesterson Reservoir is located in the San Joaquin Valley in California, where there are high concentrations of naturally occurring selenium in soils and exposed rocks. This reservoir collected runoff from irrigation and precipitation in the valley via the San Luis Drain, which was initially meant to empty into the Sacramento-San Joaquin River Delta, but was never completed. Selenium dissolved from soil and rocks in the runoff resulted in elevated selenium concentration in the water of the reservoir. Embryo and chick deformities were observed in several species of aquatic bird found in and around the reservoir (Ohlendorf, 1989). At another selenium contaminated site in California, a deformed Black-necked Stilt (*Himantopus mexicanus*) embryo, that had elevated levels of proteinaceous selenomethionine compared to normal embryos, was observed (Fan et al., 2002).

Other physiological changes have been associated with elevated levels of selenium in waterfowl. In Common Eiders (*Somateria mollisima borealis*), there was a negative correlation between corticosterone and hepatic selenium concentrations (Wayland et al., 2002). Adult Mallards exposed to 2.2 mg/L of

selenomethionine in drinking water had an altered immune response including impairment of the delayed-type hypersensitivity response. Elevated glutathione peroxidase and plasma alanine aminotransferase suggested a possibility of liver damage (Fairbrother and Fowles, 1990).

Several other selenium dosing studies, summarized in a review by Hoffman (2002), have detected altered glutathione metabolism in both plasma and liver and a corresponding increase in lipid peroxidation. These results provide evidence that selenium toxicity may be in part associated with production of the free radical, superoxide ($O_2^{-\bullet}$) during reaction with thiol containing molecules such as glutathione (Hoffman, 2002). However, proteinaceous selenomethionine, one of the forms of selenium most likely encountered by diving ducks, does not appear to produce superoxides (Spallholz and Hoffman, 2002). Two additional mechanisms of toxic action for selenium compounds may be associated with the substitution of selenomethionine for methionine in various proteins and the subsequent alteration of protein function (Spallholz and Hoffman, 2002) or the inhibition of selenium methylation by an excess of selenocysteine, resulting in accumulation of the hepatotoxic intermediate metabolite, hydrogen selenide (Sayato et al., 1997).

1.3.2 Cadmium

Cadmium, which is widely distributed through the earth's crust in mixed ores with zinc, is considered non-essential for animal nutrition (Furness, 1996). Cadmium is used in a variety of industrial processes such as plastics production, galvanizing and manufacturing of batteries (Goyer and Clarkson, 2001). Following

use in these processes, most cadmium is released and can be responsible for substantial reservoirs of cadmium in the environment (Furness, 1996). In aquatic environments, most cadmium is associated with bottom sediments but concentrations of dissolved cadmium will increase with decreasing pH (Furness, 1996). Molluscs, including Zebra Mussels, are thought to accumulate substantial quantities of cadmium during filter feeding and may represent a significant source of cadmium exposure to birds feeding on them (Furness, 1996; Berney et al., 2003). A large proportion of ingested cadmium becomes bound to the intestinal epithelium, limiting intestinal absorption of cadmium (Cd^{2+}) to approximately 0.4 to 2% of the oral dose in birds (Scheuhammer, 1987). However, the proportion absorbed may increase as a result of injury to the intestinal epithelium or deficiency of essential divalent elements such as calcium and zinc, leading to an increase in active transport of cadmium (Furness, 1996). The highest tissue concentration of cadmium in birds is almost always in the kidney (Furness, 1996). Cadmium induces production of metallothionein and, once bound to this protein, has an extremely long biological half-life (Scheuhammer, 1987). Older individuals in a population may have been exposed to cadmium for longer, and as a result of the long half-life of cadmium, have elevated tissue levels compared to younger individuals (Warren et al., 1990). Though the excretion rate of cadmium is extremely slow, some excretion does occur in the urine with lesser amounts excreted in the bile (Goyer and Clarkson, 2001).

Based on a review of laboratory and field studies, concentrations of 100

μg/g dry weight in kidney and 40 μg/g dry weight in liver have been proposed as the tentative threshold for major toxic effects of cadmium in birds (Furness, 1996), much higher than those measured in Lesser Scaup tissue (Custer et al., 2000; 2003). One of the principal sites of cadmium toxicity in birds is the kidney. Mallard ducks fed 200 μg/g of cadmium for 60 days, resulting in cadmium levels of 130-140 μg/g wet weight in kidney, developed renal tubular necrosis (White et al., 1978). Pekin ducks (*Anas platyrhynchos)* fed diets containing 50 and 300 μg/g of cadmium had increased kidney and salt gland mass, renal tubular damage and decreased glomerular filtration rate, particularly in the 300 μg/g cadmium dose group (Bennett et al., 2000). Testicular damage associated with cadmium has been documented in birds. In a captive study, Mallard males had testicular atrophy and interrupted spermatogenesis following dietary exposure to 20 and 200 ppm of cadmium for 90 days (White et al., 1978). There was delayed testicular maturation in Japanese Quail (*Coturnix coturnix japonica*) fed a diet with 75 μg/g of cadmium, which resulted in concentrations of approximately 40 μg/g wet weight in liver (Richardson et al., 1974). Other pathological changes included cardiac hypertrophy, altered structure of cells in the adrenal gland and damage to the epithelium of the small intestine. Juvenile Mallard ducks fed diets containing various concentrations of cadmium had decreased plasma triiodothyronine concentrations, increased adrenal weight and corticosterone concentrations, and a significant decrease in body mass (Di Giulio and Scanlon, 1984b). A negative correlation between body mass and cadmium was observed in wild Common Eiders (Wayland

et al., 2003).

1.3.3 Mercury

A majority of mercury in the environment is a result of degassing from the earth's crust. Human activities such as coal combustion, waste incineration, and dumping of sewage sludge can be responsible for significant additions of mercury into the environment (Thompson, 1996). Inorganic mercury exists in three oxidative states: the zero oxidative state (Hg^0) which occurs as a metal or as a vapor; the mercurous state (Hg^+); and the mercuric state (Hg^{2+}) which can form various organic forms by attaching to carbon atoms (Goyer and Clarkson, 2001). The most relevant form of mercury exposure in wildlife is organic methylmercury (CH_3Hg^+) (Thompson, 1996) formed when elemental mercury is methylated by microorganisms present in the sediments of water bodies (Goyer and Clarkson, 2001). Methylmercury enters the food chain and is transferred from microorganism through lower consumers to top level predators (Wolfe et al., 1998). Methylmercury is almost completely absorbed through the gastrointestinal tract while the intestinal absorption of inorganic forms is limited to a few percent (Goyer and Clarkson, 2001). The toxicokinetics of mercury differ for the various forms of mercury. Methylmercury and mercury vapor tend to accumulate in the brain, while kidneys tend to show highest concentrations following exposure to inorganic forms of mercury (Goyer and Clarkson, 2001). A majority of all forms of mercury is excreted through feces and urine with a small portion of inhaled mercury vapor being lost during exhalation (Goyer and Clarkson, 2001).

Methylmercury is a potent embryo and neurological toxicant producing brain lesions, spinal degeneration and altered central nervous system function (Thompson, 1996). Symptoms of acute methylmercury poisoning in birds include weight loss, weakness in limbs, an inability to coordinate muscle movements and death (Scheuhammer, 1987). Though chronic, low level dietary exposures can be lethal in birds, other effects are commonly seen (Wolfe et al., 1998). Dietary intake of 0.5 μg/g methylmercury in Mallard ducks resulted in reproductive effects including reduced hatchability, reduced clutch size (Heinz, 1975) and behavioral impairment such as decreased response to maternal warnings (Heinz, 1979). Similar behavioral and reproductive alterations have been observed in other aquatic birds when exposed to chronic dietary intakes of 0.3 to 0.4 μg/g wet weight (Scheuhammer, 1987).

Tissue concentrations of mercury have been correlated to several behavioral and physiological changes in aquatic birds. Based on a review of available literature, Zillioux et al. (1993) suggested a threshold for major toxicological effects to be 5 μg/g wet weight in avian livers, however other effects of mercury have been observed at tissue concentrations below this level. There was a negative relationship between pancreas mass and liver mercury concentrations ranging from 1.1 to 10.78 μg/g dry weight in Scaup from coastal California (Takekawa et al., 2002). Mercury concentrations from 1.5 to 9.8 μg/g dry weight in kidney were negatively related to body, heart and fat mass in nesting Common Eiders (Wayland et al., 2003). In female Mallards fed methylmercury, concentrations ranging from

0.89 to 1.62 μg/g wet weight (approximately 3.1 to 5.67 μg/g dry weight) in liver were correlated with altered laying behavior and decreased reproductive success (Heinz, 1979).

1.3.4. Interactions Between Selenium and Mercury

Although tissue mercury concentrations in Lesser Scaup from the Mississippi Flyway and the Great Lakes have typically been low, consideration of mercury is important because of considerable evidence of interactions with selenium (Scheuhammer, 1987). Typically these interactions are antagonistic though some additivity or synergism has been observed (Cuvin-Aralar and Furness, 1991). Concentrations of mercuric mercury (Hg^{2+}) were shown to decrease in the presence of sodium selenite, sodium selenate and selenomethionine in a polarographic apparatus (Feroci et al., 2005). Selenomethionine inhibited uptake of aqueous methylmercury by diatoms (*Thalassiosira pseudonana*) and green mussels (*Perna viridis*) while selenite and selenate had no effect on uptake by either species (Wang et al., 2004). Injection of sodium selenite in rats has been shown to have a protective effect against the toxic effects of mercuric chloride when injected simultaneously (Parizek and Ostadalova, 1967). Selenomethionine prevented lethal toxic effects of methylmercury chloride in adult male Mallards (Heinz and Hoffman, 1998). Interestingly, in that study, ducks treated with selenomethionine and methylmercury chloride had more reproductive toxicity (decreased hatching success, inhibited growth and increased teratogenesis) than birds treated with either selenium or mercury alone. The co-administration of mercury and various selenium

compounds in rats decreased mercury retention and damage in the kidney, and shifted mercury accumulation to the liver (Magos et al., 1987; Cikrt and Bencko, 1989). This interaction between selenium and mercury and changes in mercury distribution have been attributed to the formation of low and high molecular weight complexes of mercury and selenium (Naganuma and Imura, 1981; Yoneda and Suzuki, 1997; Cabanero et al., 2005) It appears that formation of this complex likely occurs in blood with reduced glutathione being an important component (Gailer et al., 2000).

1.4 Physiological Responses and Variables as Biomarkers

1.4.1 The Avian Stress Response

The avian stress response involves a complex interaction between nervous and endocrine systems. The hypothalamus-pituitary-adrenal axis (HPA) is considered one of the most important systems allowing birds to adapt to stressors and environmental changes (Silverin, 1998). In response to a psychologically perceived threat, the hypothalamus secretes corticotropin-releasing hormone which stimulates release of adrenocorticotropic hormone (ACTH) from the anterior pituitary (Silverin, 1998). Release of ACTH results in activation of the adrenal glands and subsequent release of corticosteroids from the cortical cells (Romero and Romero, 2002). Mammalian adrenal glands have a central medulla surrounded by the three concentric zones of the adrenal cortex and cortisol is the primary hormone released in response to stress. In contrast, corticosterone is the primary stress hormone of birds and adrenal glands do not show distinct zones, but instead

display an intermingling of cortical and medullary cell clusters (Ghosh et al., 2001).

In response to an acute stressor, such as agonistic interactions with a conspecific, threat from a predator, or capture and handling, there is a rapid rise of circulating corticosterone. This rise in hormone level stimulates other physiological and behavioral changes (Wingfield et al., 1982; Gratto-Trevor et al., 1991; Romero and Romero, 2002). Glucocorticoids, including corticosterone, stimulate mobilization of lipid reserves and conversion of circulating triglycerides to glucose during gluconeogenesis (Remage-Healey and Romero, 2001). This process provides additional energy stores which are used during attempts to resolve the stressful situation (Carsia and Harvey, 2000) Other characteristic physiological responses to acute stressors include an increase in body temperature and an elevation of heart rate (Cabanac and Guillemette, 2001) though these responses are more likely associated with catecholamines released from the adrenal medulla during the sympathetic-adrenal medullary system activation (Silverin, 1998).

Several avian studies have used corticosterone levels as a measure of stress. Clear interpretation of results, however, are often confounded by the degree of variation among individuals and studies (Silverin, 1998). Several factors could be responsible for this variation in birds. There is significant daily and seasonal variation in both basal and stress-induced levels of corticosterone in several bird species (Silverin, 1998; Remage-Healey and Romero, 2001; Landys et al., 2004). Environmental factors such as breeding latitude may also affect the stress response. For example, high arctic breeding birds have lower corticosterone secretion in

response to capture and handling compared to low arctic breeding birds (Silverin, 1998; O'Reilly and Wingfield, 2001). There is considerable evidence that individuals at different positions within a social hierarchy respond differently to stressors (Kotrschal et al., 1998; Senar et al., 2000; Poisbleau et al., 2005b). Though these differences may not be reflected in baseline corticosterone levels, there may be significant differences in corticosterone response at subsequent sampling times between subordinate and dominant individuals (Silverin, 1998; Poisbleau et al., 2005b). Contaminants may contribute additional variability to the stress response of an individual by altering adrenal structure or function, thus influencing circulating levels of corticosterone (Richardson et al., 1974; Di Giulio and Scanlon, 1984b; Colby et al., 1997; Capen, 2001; Wayland et al., 2002; Mayne et al., 2004).

Significant variability can also be introduced by trapping and capturing activities, especially when attempting to establish baseline corticosterone concentrations in wild birds. Birds left in mist nets for 15 minutes following capture, had significantly elevated baseline corticosterone levels compared to birds removed immediately from nets (Romero and Romero). The subsequent blood samples did not show significant variation of corticosterone levels between individuals however. This research shows the importance of rapid sampling of individuals when attempting to establish baseline corticosterone levels. This study also showed, that individuals trapped using Potter traps exhibited no variation in baseline corticosterone levels between birds left in traps for 15 minutes before

sampling and those sampled immediately after capture. This may provide evidence of the influence of trapping technique on the psychologically perceived threat by trapped birds and resulting stress response. Less threatening trapping techniques, such as potter traps, may reduce variability introduced by inconsistent time between capture and sampling.

Despite the avian stress response being an evolved trait meant to allow an organism to deal with and resolve adverse or threatening situations, prolonged exposure to stressors can have a marked deleterious effect on an individual's physiology. Increased production of glucose and the resulting increase of blood glucose levels in response to stress can lead to decreased glucose carbons being integrated into proteins (Siegel, 1980). A prolonged increase of corticosteroid levels can inhibit calcification of the skeleton during growth or induce osteoporosis in adult birds (Siegel, 1980). Elevated corticosterone levels have been associated with decreased levels of reproductive hormones in birds which can result in decreased gonadal function and development (Silverin, 1998).

Chronic stress may also influence thyroid hormone levels. The avian thyroid secretes two primary hormones, tetraiodothyronine or thyroxine (T_4) and triiodothyronine (T_3). Thyroxine is converted to biologically active triiodothyronine by the action of deiodinase enzymes. Thyroid hormones have multiple functions in birds including regulation of metabolic rate and stimulation of growth and differentiation during development (Decuypere et al., 2005). Repeated exposure to stressors and glucocorticoids has been shown to affect thyroid hormone levels in

several species (Kuhn et al. 1998). Chronic corticosterone administration caused decreased circulating plasma thyroid hormone levels in chickens (Williamson and Davidson, 1987). Repeated exposure to stressors for 15 days resulted in decreased serum thyroxine and triiodothyrinine levels in rats (Helmreich et al. 2005). A similar reduction in thyroid hormone levels was observed in mice exposed to chronic mild stress for six weeks (Silberman et al. 2002).

Increased corticosterone concentration has also been shown to alter immune response in birds (Siegel, 1980; Dohms and Metz, 1991; Martin et al., 2005). Artificially elevated levels of corticosterone suppressed the skin swelling response of House Sparrows (*Passer domesticus*) when injected with phytohemagglutinin (Martin et al., 2005). This suppression of cutaneous immune function in response to chronically elevated corticosterone may be an evolved mechanism to limit the nutritional and energetic cost of immune activity or minimize autoimmune damage (Sapolsky et al., 2000). A significant positive correlation between increasing corticosterone and number of heterophils relative to lymphocyte numbers has been observed in several species of bird (Siegel, 1980; Vleck et al., 2000; Vleck, 2001). It has been proposed that this is part of an evolved mechanism in which elevated corticosterone decreases lymphocyte numbers, limiting release of inflammatory cytokines and preventing excessive inflammation of tissue damaged during the resolution of a stressful encounter (Vleck, 2001). Application of exogenous corticosterone to chickens, however, causes histopathological changes and atrophy of lymphoid tissues, indicating that chronic elevation of corticosteroids due to

prolonged bouts of stress may cause more permanent immunological impairment, which could result in decreased resistence to viruses, bacteria or parasites (Siegel, 1980; Dohms and Metz, 1991).

Based on the above studies, it is clear that when attempting to compare stress response between individuals of a population, attempts should be made to account for and limit the degree of variability in results by standardizing the length of time from capture to sample completion, the daily and seasonal sampling time and location, as well as identifying dominant and subordinate individuals for separate analysis due to possible differences in their physiological response to stress. Additionally, measurement of several variables associated with stress response and the resulting physiological changes may serve to clarify differences between individuals and possible influences of contaminants on stress related variables.

1.4.2 Male Reproductive Readiness

Reproductive success and development relies on a complex interaction of hormonal, environmental and behavioral variables. Spermatogenesis and seasonal reproductive development in birds, though partially mediated by cells in the testis, is ultimately controlled by the central nervous system (Sharp and Gow, 1983). Nervous system activation in turn, is stimulated by environmental factors, the most important of which appears to be seasonal cycles of daily photoperiod (Sharp et al., 1986; Wingfield et al., 1992). Photoreceptors responsible for nervous system stimulation are located in the hypothalamus and lobus parolfactorius in the ventral

forebrain (Kuenzel, 1993). Following stimulation of the central nervous system by these photoreceptors, gonadotropin-releasing hormone is secreted from the hypothalamus, causing release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (Sharp and Gow, 1983; Bluhm, 1992). These two hormones stimulate gonadal development by interacting directly with cells in the testes. Follicle-stimulating hormone induces development and proliferation of Sertoli cells. These cells stretch from the basal lamina to the lumen of the seminiferous tubules and primarily function to provide a regulated environment for germ cell development (Kirby and Froman, 2000). Luteinizing hormone stimulates the action of Leydig cells located outside seminiferous tubules in the testicular interstitium (Aire, 1997). The primary function of Leydig cells is production and secretion of gonadal steroid hormones (Farner and Wingfield, 1980).

Testosterone is the primary reproductive steroid secreted from Leydig cells and is partially responsible for maintenance and development of several reproductive activities including initiation of spermatogenesis and development of the reproductive tract (Bluhm, 1992). Spermatogenesis begins with a series of mitotic divisions resulting in an increase in the number of spermatogonia at the seminiferous tubule basement membrane. This is followed by primary spermatocyte formation and its subsequent separation from the basement membrane. Following separation, the spermatocyte begins active meiosis creating several types of spermatocytes as it moves towards the center of the seminiferous tubule. The final

meiotic division leads to formation of spermatids which undergo spermiogenesis, resulting in formation of sperm cells at the lumen of the seminiferous tubule (Kirby and Froman, 2000).

A seasonal increase in testosterone secretion stimulates a variety of reproductive related behaviors including courtship displays and increased competitiveness and aggression (Bluhm, 1992). A positive correlation has been observed between testosterone levels, pair status and incidence of extra-pair copulations in Mallards (Davis, 2002). Though testosterone is thought to be the primary hormone regulating these reproductive behaviors, there is evidence that other hormones including corticosterone play an important role in pair formation, levels of aggression and the defense of a mate following pairing in waterfowl (Sorenson et al., 1997).

It has been suggested that Lesser Scaup population declines may be related to a decline of reproductive success (Austin et al., 2000). As a result, an assessment of the impact of contaminants on Lesser Scaup reproductive success and behavior was recommended. Though a majority of research on effects of contaminants on reproductive success focuses on females, contaminants can also negatively affect function, stimulation and development of the male reproductive system (Richardson et al., 1974; White et al., 1978). This could, in turn, influence breeding related behaviors and success in males. Corticosterone and stress can adversely affect reproductive parameters in birds (Silverin, 1998) and, in turn, also be affected by contaminant exposure. These complex interactions of hormones,

physiology, behavior, and contaminants need to be examined to more clearly understand possible influences of contaminants on stress response and reproductive readiness in male Lesser scaup.

Hypothesis:

Trace element contaminants can alter hormonal status in waterfowl and in turn, alter related physiological processes including stress response and seasonal reproductive development. Expression of toxic effects on hormonal status can be modified by interactions between different contaminants and other variables (physiological, environmental, social).

1.5 Primary objectives

- Assess the impacts of trace element contaminants on stress response of paired and unpaired male Lesser Scaup from the northern boreal forest
- Characterize reproductive readiness of paired and unpaired male Lesser Scaup, and assess the impact of trace element contaminants on these variables
- Investigate dose-response relationship between selenium and corticosterone in a controlled environment using captive male Lesser Scaup

2.0 Stress and Trace Element Contaminants in Male Lesser Scaup (*Aythya affinis*)

2.1 Introduction

The stress response of animals is an evolved response meant to help cope with a variety of stressors including changes in weather conditions or food resources, threats from predators, and interactions with conspecifics (Silverin, 1998). Hypothalamus-pituitary-adrenal axis activation and subsequent release of corticosterone is considered one of the characteristic responses to stress in birds (Romero and Romero, 2002). The release of corticosterone stimulates other changes including immune system activation and altered behavioral patterns (Siegel, 1980; Silverin, 1998; Sapolsky et al., 2000). Corticosterone release also stimulates gluconeogenesis resulting in a rapid increase in circulating levels of glucose following exposure to a stressor (Siegel, 1980; Sapolsky et al., 2000). This mobilization of energy provides resources needed to deal with injury, escape or resolve a stressful situation (Siegel, 1980).

Many studies have measured corticosterone to characterize causes and

consequences of stress in birds, though clear interpretation of results is often confounded by variation among individuals and studies (Silverin, 1998). Corticosterone can be influenced by time of day, season, social status and body condition (Remage-Healey and Romero, 2001; O'Reilly and Wingfield, 2001, Kotrschal et al., 1998; Senar et al., 2000; Poisbleau et al., 2005b; Perfito et al., 2002).

Contaminants and trace elements can influence corticosterone levels in birds. For instance, corticosterone concentrations decreased in relation to increasing liver concentrations of selenium in Eiders (Wayland et al., 2002) while a positive relationship was observed with kidney cadmium in Eiders (Wayland et al., 2002) and blood lead levels in Storks (Baos et al., 2006). These studies have typically considered the effects of individual contaminants or physiological variables on corticosterone secretions. There is considerable evidence, however, that trace elements and metal contaminants can interact, modifying the expression of toxic effects. Selenium is an important component of antioxidant defenses in the body, and has been shown to have a protective effect against the oxidative damage associated with heavy metal contaminants (Cuvin-Aralar and Furness, 1991; Shaikh et al., 1999; Yiin et al., 1999). Selenium can form complexes with metals or alter distribution of toxic metals in tissues, further limiting the effects associated with both metals contaminants and toxic levels of selenium (Cuvin-Aralar and Furness, 1991; Sasakura and Suzuki, 1998; Cabanero et al., 2006).

Though elevation of corticosterone in response to a stressor can be

beneficial in resolving a situation in the short term, prolonged exposure to glucocorticoids can have a negative effect on an individual. Chronic exposure to corticosterone has been associated with depressed growth and suppressed immune function (Siegel, 1980). Chronic stress can also suppress levels of other hormones including reproductive and thyroid hormones (Williamson and Davidson, 1987; Kuhn et al. 1998; Sapolsky et al., 2000). Though the primary hormone secreted from the thyroid gland is thyroxine (T_4) the most biologically active thyroid hormone is triiodothyronine (T_3). Most circulating T_3 likely originates from the conversion of T_4 to T_3 by enzymes found in liver, kidney and brain (Leatherland, 2000). Thyroid hormones have multiple functions in birds including stimulation of growth and development and regulation of metabolic rate (Decuypere et al., 2005). Exposure to acute and chronic stress has been shown to suppress circulating T_4 and T_3 levels (Decuypere et al., 2005; Helmreich et al., 2005), though changes in T_4 levels in response to stress have been somewhat variable in birds (Kuhn et al., 1998).

Concerns have recently been raised regarding potential effects of contaminants on Lesser Scaup (*Aythya affinis*) populations (Austin et al., 2000). Lesser Scaup is one of the most common North American diving ducks with a broad distribution throughout much of the continent (Austin, 1998). There have been dramatic declines in the combined Greater (*Aythya marila*) and Lesser Scaup populations since the 1970s, to a record low of 3.39 million birds in 2005 (NAWMP, 2004, Ducks Unlimited, 2005). Accompanying the population decline

have been two additional shifts in Lesser Scaup demographics: a decrease in the proportion of young birds which may indicate a decrease in reproductive success and an increase in the ratio of males to females. Declines appear most severe in the boreal forest region of western Canada (Austin et al., 2000) which is the core breeding habitat for Lesser Scaup (Austin et al., 1998). During migration to breeding grounds, large numbers of Scaup stage and migrate through highly contaminated sites on the Great Lakes. The number of Scaup wintering and staging on the Great Lakes appears to be increasing, despite overall population declines, likely in response to proliferation of easily accessible food sources including Zebra Mussels (*Driessena polymorpha*) (Petrie and Schummer, 2002). Though these mussels provide an easily accessible food source for staging Scaup, they may represent a significant source of contaminant exposure (Petrie, 2004).

Several studies have assessed contaminant levels in Lesser Scaup from various sites throughout the Great Lakes and the Mississippi Flyway (Custer and Custer, 2000; Custer et al., 2000; Custer et al. 2003; Petrie, 2004). Most of the contaminants measured (including Cd, Pb and Hg) have been found at levels below those known to cause major toxic effects. Selenium has become the primary contaminant of concern for Lesser Scaup populations (Custer et al., 2003). Selenium has been consistently detected at potentially harmful levels in Lesser Scaup and Zebra Mussels from the Great Lakes (Custer and Custer, 2000; Custer et al., 2000; Custer et al. 2003; Petrie, 2004). Concentrations of approximately 10 μg/g dry weight in livers may be associated with reproductive impairment in

waterfowl, while impacts on animal health and survival may be seen at concentrations of 35 μg/g dry weight (Heinz, 1996). Anthropogenic sources, such as fly ash from coal power plants, mining of metal ores and runoff from agricultural activities are likely responsible for elevated levels found in the Great Lakes (Hoffman, 2002). Selenium can enter the foodweb resulting in trophic transfer of elevated levels of selenium from lower organisms such as zooplankton, through invertebrates such as bivalves, to upper level consumers (Ohlendorf, 1989; Fan et al., 2002). Trophic transfer of selenium through Zebra Mussels is likely responsible for elevated concentrations of selenium observed in Lesser Scaup from the Great Lakes region (Petrie, 2004). Although tissue selenium levels in waterfowl rapidly increase in the presence of a contaminated food source, they also decline rapidly when diet is shifted to less contaminated food sources (Heinz, 1996). At present, there is little contaminant data for Lesser Scaup from sites in the boreal forest and, as a result, it is not known whether elevated concentrations persist through migration to affect individuals on breeding grounds.

There were two primary objectives to this portion of the study. The first was to examine the relationship between tissue contaminant concentrations and stress-related variables (corticosterone, glucose and thyroxine) in male Lesser Scaup from breeding grounds in the boreal forest. This would test the hypothesis that trace element contaminants affect hormone levels and alter response to stressors. The second objective was to examine the stress response of paired and unpaired male Lesser Scaup to understand possible implications of an increase in

the male sex ratio bias. To fully characterize the stress response, the effects of contaminants, and differences between paired and unpaired males, several variables related to stress were measured including corticosterone levels, mobilization of energy reserves as reflected in blood glucose levels and thyroid hormone status.

2.2 Materials and Methods

2.2.1 Study Site

Field work was conducted in the boreal forest in May and June along the Ingraham Trail, approximately 25 km east of Yellowknife, Northwest Territories, Canada (62° 31' 00" North, 114° 11' 00" East). In 2004, Lesser Scaup were trapped between June 4 and June 21 on a single unnamed lake along the Ingraham Trail. Between May 21 and June 6 in 2005, trapping was continued on the unnamed lake and expanded to Tom Lake located approximately 1 km east of the 2004 site. More Lesser Scaup were seen on these two bodies of water than on any other wetland along the Ingraham trail.

2.2.2 Field Procedure and Sampling

Lesser Scaup were captured using decoy traps (Anderson et al., 1980) with a Lesser Scaup female placed in the centre compartment of traps. Animals were treated in accordance with Canadian Council on Animal Care guidelines and approved protocols. Pair status of Lesser Scaup males captured in traps was identified in one of three ways: 1) Capture, marking, observation and recapture; 2) Direct observation during trapping; 3) Assumption based presence or absence of female in compartment with male. In 2004, the primary method was the marking

and recapture procedure. This involved attaching unique combinations of vinyl nasal tags to captured males. Males were then released and observation was used to re-sight and identify pairs status based on interactions with females. Recapture rate of marked individuals was low, requiring modification to pair status identification protocol. In 2005, pair status determination focused on direct observation and identification of pair status as individuals entered traps. This eliminated the need for marking, release and recapture. Limited field crew size prevented continuous trap monitoring, however, so when not under direct observation, males captured in the same compartment as a female in an otherwise empty trap, were identified as paired males. Those males captured alone in compartments were tentatively identified as unpaired, banded with United States Geological Survey bands and released.

When males of known pair status were captured, time of day was recorded. As traps were approached, the time when captured males first showed signs of disturbance due to approach of researchers (i.e., wing flapping, attempts to escape) was recorded. When removed from the trap, Scaup underwent a standardized capture and handling procedure known to cause a rapid increase in circulating corticosterone (Wingfield et al., 1982). Approximately 1 ml of blood was collected via jugular venipuncture immediately following capture. The time from initial disturbance to completion of sample was recorded. Each sample was placed in a serum separator vacutainer, and was stored in a cooler with ice packs until being centrifuged at 3000 rpm for 10 minutes. Tubes were then frozen at -20°C until

analysis could be done. Males of known pair status were euthanized by $C0_2$ inhalation or cervical dislocation, weighed to the nearest 10 g and morphometric measurements (head to bill, wing cord and tarsal lengths) were recorded. Carcasses were held frozen at -20°C until thawed for dissection.

2.2.3 Tissue Collection and Processing

During dissection, liver and kidneys were removed. A 5 g (±0.1 g) subsample of liver was taken and frozen at -20°C for selenium and mercury analysis. The remaining liver was weighed to the nearest 0.1 g and then freeze-dried (Benchtop freeze dry system, Labconco, KC, MO, USA) for 3 to 4 days until a consistent weight was obtained. The kidneys were weighed to the nearest 0.1 gram and then freeze-dried. Moisture content of freeze-dried tissue was calculated using the formula: % moisture = 100 - (W_d/W_w) x 100, where W_d = weight of dry sample and W_w = weight of wet sample (Neugebauer et al., 2000).

2.2.4 Contaminant Analysis

Frozen liver samples were submitted to the University of Saskatchewan Prairie Diagnostic Service Toxicology lab (Western College of Veterinary Medicine, Saskatoon, SK) for selenium and total mercury residue analysis. Freeze dried kidneys were analyzed for cadmium residue at the University of Saskatchewan Toxicology Centre (Saskatoon, SK). All tissues were digested in 69-70% nitric acid using a microwave accelerator reactor system (CEM Corp., Matthews, NC, USA). Selenium was measured using an inductively coupled plasma-emission spectrophotometer equipped with a hydride generator accessory (Thermo Jarrel

Ash Corp, Franklin, MA, USA) (Hoffman et al., 1998). Total mercury was measured using an inductively coupled plasma-mass spectrophotometer (Thermoelectron Corp., Chesire, United Kingdom) (Shuqin et al., 1999). Cadmium was measured using a Varian SpectrAA 220Z graphite furnace equipped with a graphite tub atomizer (GTA-110Z) with Zeeman background correction (Berney et al., 2003). Lobster hepatopancreas (TORT-2) (National Research Council of Canada) was used as certified reference material for cadmium analysis with recoveries between 85 and 115%.

2.2.5 Hormone and Glucose Analysis

Hormone analyses were conducted by the University of Saskatchewan Prairie Diagnostic Service Endocrinology lab at the Western College of Veterinary Medicine, Saskatoon, SK. Serum corticosterone concentration was measured using a corticosterone ^{125}I radioimmunoassay (RIA) kit (MP Biomedicals, LLC, Orangeburg, NY, USA) (Sorenson et al., 1997). The manufacturer's protocol was followed except that serum samples were diluted 1:100 rather than 1:200. Intra-assay coefficients of variation were 6.4% and 3.3% with means of 76.9 and 522.6 ng/ml respectively. Total (bound and unbound) serum thyroxine (T_4) concentrations were measured using a Coat-a-Count canine T_4 radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA, USA) (Fernie et al., 2005). The manufacturer's protocol was followed except that 50 µl of sample was used in the assay instead of the recommended 25 µl. Intra-assay coefficients of variation were 3.4% and 6.8% with means of 57.9 and 18.8 μg/dl respectively. Serum glucose

concentrations were measured at the University of Saskatchewan Toxicology Centre using a glucose oxidase/peroxidase (PGO) assay and methods described by the manufacturer (Sigma-Aldritch, Inc, Saint Louis, MO, USA).

2.2.6 Statistics

All data were analyzed using SPSS 14.0 (SPSS Inc. 2005). Disproportionate samples from 2004 and 2005, especially in paired individuals, prevented meaningful comparisons between year. Unpaired birds were compared and showed few differences with the exception of body size (t-test, df=18 P=0.02) and thyroxine concentrations (t-test, df=18, P=0.03). Although not ideal, data for both years was lumped for analysis and, where possible, differences are highlighted in the following text. Morphometric measurements (head to bill length, wing cord and tarsal length) were used to derive an index of body size using principal component analysis. The first axis of the principal component analysis (PC1) explained 49.4% (λ_1=1.48) of variability in structural size. The component loading of morphometric measurements for PC1 were wingchord (-0.002), tarsus length (0.581), and head to bill length (0.581). Surprisingly, wingchord length had a negative component loading indicating that larger birds had smaller wingchord length. Body mass was regressed against PC1 scores to correct for body size (R^2=0.292, df=38, P<0.001) and residuals from this relationship were used as an index of body condition.

Serum corticosterone and glucose levels were highly correlated with the time interval between completion of blood sample and when disturbance was first

observed in males. To control for sampling time, corticosterone and glucose levels were regressed against time and the residuals were used in subsequent analysis.

Prior to further analysis, all data were evaluated for normality using a Kolmogorov-Smirnov test and where necessary, were log-transformed to normalize data. Tests for normality showed non-normal distributions for liver selenium and kidney cadmium and values were therefore $\log_{10}$-transformed prior to further analysis.

General linear models (GLM) were used to examine the effects of body condition and size, contaminants, pair status and collection date on corticosterone, glucose and thyroxine levels. Analyses were limited to two-way, *a priori* interactions with the exception of the three-way interaction of total contaminant load of the trace elements considered. Modeling procedure followed that described by Alisauskas and Ankney (1994) where non-significant terms ($P>0.10$) were systematically removed from significant models ($P<0.05$). Several interactions remained in the corticosterone model and interactions were therefore further broken down to arrive at simpler models and aid in interpretation.

2.3 Results

In 2004, 11 unpaired and 2 paired Lesser Scaup males were collected between June 4 and June 21 using the marking and recapture protocol. In 2005, 9 unpaired and 17 paired males were collected between May 21 and June 6, bringing total sample size to 39 males.

2.3.1 Trace Element and Contaminant Levels

The geometric mean concentrations of kidney cadmium, and liver selenium and mercury were 9 μg/g, 4.33 μg/g, 1.31 μg/g dry weight respectively. Mean values, range and minimum and maximum values are reported in Table 2.1.

2.3.2 Corticosterone

Examination of effects of pair status, body condition, body size, collection date and contaminants produced a highly significant model (R^2=0.746, $F_{18,20}$=6.350, P<0.0001) (Table 2.3) containing numerous interaction terms. Body condition, body size and pair status interacted with cadmium (Table 2.3). Therefore, to understand these complex biological relationships, birds were grouped into high and low cadmium groups based on the median cadmium value. After analysis, the high cadmium model ($F_{6,12}$=10.509, P<0.001) contained two interactions, selenium × body condition ($F_{1,17}$=12.745, P=0.004) and mercury × body size ($F_{1,17}$=17.836, P=0.001). To better understand these relationships, body condition and body size were separated based on median values. Birds with high cadmium and good body condition had a negative relationship between liver selenium and corticosterone (R^2=0.701, n=9, P=0.005) (Fig. 2.1a). In individuals with high cadmium and poor body condition, there was no relationship (R^2=0.033, n=10, P=0.614) between liver selenium and corticosterone (Fig. 2.1b). Large sized birds with high cadmium had a positive but non-significant trend between liver mercury and corticosterone (R^2=0.307, n=9, P=0.121) (Fig. 2.1c). However, in small sized individuals a significant negative relationship (R^2=0.491, n=10, P=0.024) between mercury and corticosterone existed (Fig. 2.1d).

After analysis, the selenium × mercury ($F_{1,16}$=16.802, P=0.001) interaction remained important in the low cadmium model ($F_{4,16}$=6.978, P=0.003). To better understand this relationship, birds were grouped into high and low mercury individuals based on median values. In individuals with low cadmium and high mercury, there was a positive, though not statistically significant trend (R^2=0.325, n=10, P=0.085) between selenium and corticosterone (Fig. 2.2a). In individuals with low cadmium and mercury concentrations, there was negative relationship (R^2=0.568, n=10, P=0.012) between liver selenium and corticosterone (Fig. 2.2b).

Figure 2.3 depicts the interaction of cadmium and pair status in relation to corticosterone concentration. When the high and low cadmium groups were examined separately, corticosterone concentrations was greater in unpaired birds than paired birds in the low cadmium group ($F_{1,16}$=6.696, P=0.023) while no significant difference was observed between paired and unpaired birds in the high cadmium group.

2.3.3 Glucose

The possible effects of contaminants, pair status, collection date, body condition, body size and a number of interaction terms on glucose (Table 2.2) were considered. The resultant model however, was not significant (R^2=0.551, $F_{21,38}$=0.993, P=0.513).

2.3.4 Thyroxine

Examination of effects of pair status, body condition, body size, collection date and contaminants on thyroxine produced a significant model (R^2=0.622,

$F_{4,34}$=13.98, P<0.001) (Table 2.4). Mercury concentrations in paired individuals had a positive relationship to thyroxine concentrations (R^2=0.485, n=19, P<0.001) (Figure 2.4) while no such relationship occurred in unpaired individuals (R^2=0.063, n=20, P=0.284) (Fig. 2.4). Date was also associated with thyroxine levels ($F_{1,27}$=6.775, P=0.014). Sampling occurred earlier in 2005, with birds showing higher thyroxine levels than those sampled later in the year in 2004. This year effect could be associated with uneven samples of paired and unpaired birds in 2004 and 2005 since paired birds had higher thyroxine levels than unpaired birds (t-test, df=37, P<0.001).

2.4 Discussion

2.4.1 Trace Elements and Contaminants

In this study, 21 of 39 (54%) ducks had kidney cadmium concentrations above levels considered background for waterfowl (7 µg/g dry weight) (Puls, 1994), though none were above the 100 µg/g dry weight threshold for major toxic effects (Furness, 1996). Lesser Scaup males had liver selenium concentrations ranging from 2.12 to 12.72 μg/g dry weight with only one exceeding 10 µg/g dry weight, which is the level shown to have caused reproductive impairment in females (Heinz, 1996). Concerns about the effects of elevated selenium on reproductive success have focused on females, due to deposition of selenium in eggs which can lead to malformation of developing embryos. Males tissue levels, however, may be better indicators of maximum selenium in Lesser Scaup on the breeding grounds since tissue selenium levels in female birds can decrease as selenium is deposited in

developing eggs. If females have similar selenium levels to the males in this study, it is possible females may also arrive on breeding grounds in the Northwest Territories with selenium levels which could affect reproduction due to deposition in eggs. Mercury concentrations in all cases were below threshold for major toxic effects in birds (15 μg/g dry weight) (Zillioux et al., 1993).

2.4.2 Corticosterone

Results from this study show that corticosterone release in response to acute stress (i.e., handling) is influenced by a complex array of contaminant interactions with pair status and body condition and size. Kidney cadmium concentrations appeared to have considerable influence on corticosterone secretions in my sample. Other studies have also observed the influence of cadmium on corticosterone secretion. Cadmium levels have previously been found to have a positive relationship with corticosterone levels (Hidalgo and Armario, 1987; Wayland et al., 2002). This increase could be a result of direct interaction of cadmium with adrenal glands or through interaction with other tissues in the hypothalamic-pituitary-adrenal axis (Hidalgo and Armario, 1987). Cadmium accumulates in a variety of tissues including the hypothalamus and pituitary (Clark et al., 1985; Lafuente and Esquifino, 1999; Lafuente et al., 2000). Cadmium exposure can alter pituitary function resulting in elevation of adrenocorticotropin hormone (ACTH) levels (Hidalgo and Armario, 1987). This elevation of ACTH could cause stimulation of adrenal glands and corticosterone release, explaining the positive association between cadmium and corticosterone.

In the present study, when cadmium concentrations were high, effects of selenium and mercury on corticosterone were modified. Lesser Scaup males with high cadmium had differing relationships between selenium and corticosterone (Fig. 2.1a and b) depending on their body condition (i.e., good or poor). Another study of Scaup has also found an association between cadmium, selenium and measures of body condition (i.e. lipid reserves), with cadmium being negatively correlated to body condition and selenium showing a positive association to body condition (Anteau et al., 2007). Metallothionein is a small protein important for binding both essential and toxic metals. Cadmium is a potent inducer of metallothionein and, once bound to this protein, become sequestered in tissues such as the kidney, where it is no longer bioavailable to exert toxic effects (Whanger and Oh, 1979). Nutrient deficiencies (i.e., sulfur) may limit synthesis of proteins including metallothionein (Whanger and Oh, 1979). In individuals in good body condition, adequate nutrition may allow greater metallothionein synthesis resulting, in more bound cadmium, limiting the cadmium-mediated increase in corticosterone levels. Thus, a negative correlation is observed between selenium and corticosterone in individuals with good body condition.

Selenium has been shown to have a negative association with corticosterone in other studies. Selenium reduced corticosterone and increased cortisol levels in adrenal glands of seals, suggesting that selenium altered steroid synthesis (Freeman and Sangalang, 1977). The suggestion that selenium inhibits steroid synthesis was supported by the negative correlation between selenium and corticosterone in

Common Eiders (Wayland et al., 2002). However, this correlation was only observed in females and not males.

Individuals with high cadmium and poor body condition did not have a negative relationship between selenium and corticosterone (Fig. 2.1b). Lower metallothionein levels could allow greater expression of a cadmium induced rise in corticosterone, eliminating the negative correlation between selenium and corticosterone. In other waterfowl species, individuals that were feed-restricted and exposed to cadmium had higher corticosterone levels than those unexposed birds (Di Giulio and Scanlon, 1985; Kitaysky et al., 2001; Wayland et al., 2002). Poor condition may indicate a degree of feed restriction and when combined with possible decreased metallothionein synthesis, may result in a cadmium-induced rise in corticosterone and limit suppression of corticosterone by selenium.

Males with high levels of cadmium also had a differing relationship between mercury and corticosterone (Fig. 2.1c and d) depending on their body size. Poor nutrition early in life can affect growth rates in young birds and leading to smaller body size in adults (Searcy et al., 2004). Nutrition can also influence development and growth of internal organs with poor nutritional diets leading to smaller organs (Burrin et al., 1990; Nyachoti et al., 2000; Fortman et al., 2005). Body size has been associated with differences in adrenal activity and stress response with smaller individuals typically showing higher levels of cortisol (Riemer et al., 1990; Atkinson et al., 1995).

In males with high cadmium and small body size, there was a negative

relationship between mercury and corticosterone (Fig. 2.1d). Blood mercury concentrations above 4 μg/g wet weight have been associated with elevated corticosterone levels in Common Loons (*Gavier immer*) (Burgess et al., 2005). Exposure to mercury can also result in increased cortisol secretions in fish (Bleau et al., 1996). Other studies however, have reported no correlation between tissue mercury concentrations and corticosterone (Wayland et al., 2002; 2003; Heath and Frederick, 2005). In contrast, in this study corticosterone concentrations were lower in small-sized individuals with high cadmium when mercury concentrations increase (Fig. 2.1d). To my knowledge there are no other studies that have considered effects of body size, cadmium and mercury on corticosterone secretions. Differences in body size of unpaired birds from 2004 and 2005 may also have contributed to the significance of body size in the corticosterone model and results should therefore be interpreted cautiously. Further research is needed to understand the implications and mechanisms of these relationships.

Lesser Scaup males with low cadmium had a different relationship between selenium and corticosterone (Fig. 2.2a and b), depending on high or low mercury levels. There is considerable information regarding the interaction between selenium and mercury. Typically these interactions are antagonistic, decreasing expression of toxic effects (Cuvin-Aralar and Furness, 1991; Heinz and Hoffman, 1998). The detoxification could be related to several possible mechanisms including the redistribution of mercury to less sensitive tissue, prevention of oxidative damage by selenium based antioxidants, competition for binding sites

between the two elements, or the formation of a mercury and selenium complex (Cuvin-Aralar and Furness, 1991; Cabanero et al., 2006). Complexes form, likely in association with glutathione molecules, binding the two elements in equimolar ratios (Cabanero et al., 2006). The formation of complexes likely reduces bioavailability of the two elements, thereby reducing the ability to cause toxic effects. When mercury is low, few Se-Hg complexes are formed leaving selenium free to influence corticosterone secretions. Thus, in this study, when both cadmium and mercury were low, the negative association between selenium and corticosterone was evident. This negative correlation observed between selenium and corticosterone is similar to that found in other studies (Freeman and Sangalang, 1977; Wayland et al., 2002).

In birds with low cadmium and high mercury, formation of Se-Hg complexes likely occurs, limiting the ability of selenium to exert negative effects on corticosterone levels. This may allow a slight positive influence by cadmium on corticosterone to be observed (Fig. 2.2a), similar to that seen in a previous study (Wayland et al., 2002).

Cadmium concentrations also had a differing relationship with corticosterone levels in individuals of different pair status (Fig. 2.3). In birds with high cadmium, there was no difference in corticosterone levels between individuals of different pair status. High cadmium may be stimulating corticosterone release, thus obscuring any differences in corticosterone response associated with pair status. In birds with low cadmium levels however, unpaired males had higher

corticosterone than paired birds. These results are consistent with other studies in which unpaired or subordinate birds had higher levels of corticosterone than paired birds. Subordinate individuals had higher adrenocortical response to stress than dominant individuals in White-throated sparrows (*Zonotrichia albicollis*) and Blue-footed boobies (*Sula nebouxii*) (Silverin, 1998). Unpaired geese during the mating season also had higher levels of fecal corticosterone levels than paired individuals (Kotrschal et al., 1998). Unpaired birds may be experiencing a greater degree of stress during the mating season due to limited access to females, unsuccessful attempts to acquire mates, or because they are more frequent targets for aggression from dominant males (Kotrschal et al., 1998). During periods of parental care however, paired male geese with offspring had higher levels of corticosterone that unpaired males or paired males with no offspring. This seems to indicate a seasonal or annual cycle of social stress and may help to explain variable results in other studies where subordinate individuals had lower corticosterone response to stress than dominant individuals (Kotrschal et al., 1998; Silverin, 1998; Poisbleau et al., 2005b).

2.4.3 Glucose

In this study, glucose levels did not appear to be affected by contaminants. Other studies, have shown glucose concentrations can be affected by contaminant levels. Exposure to mercuric chloride and methylmercury resulted in an increase in plasma glucose levels in fish (Bleau et al., 1996). Acute exposure to selenium also caused a rise in plasma glucose in rats (Potmis et al., 1993). Acute cadmium

exposure also caused elevated levels of glucose in carp (Drastichova et al., 2004). Chronic exposure to cadmium however, caused a decrease in plasma levels of glucose in trout (Chowdhury et al., 2004) while the opposite was observed in mammals (Rajanna et al., 1984).

2.4.4 Thyroxine

Thyroxine levels were influenced by an interactions between pair status and mercury as well as by the date captured. There was a significant positive correlation between mercury and thyroxine concentrations in paired individuals (Fig. 2.4). In unpaired individuals, there was no correlation observed between mercury and thyroxine. Exposure to various forms of mercury has been associated with elevation of thyroxine levels (Barregard et al., 1994; Bleau et al., 1996). It has been suggested that mercury in the liver may inhibit the action of deiodinase enzymes thereby limiting the conversion of thyroxine to triiodothyronine (Sin et al., 1990). This could result in higher thyroxine levels in individuals with higher liver mercury levels. The highest mercury concentrations in my sample were seen in paired individuals and this could account for the positive correlation with thyroxine levels in paired birds (Figure 2.4).

Prolonged exposure to stress and elevated corticosterone can decrease thyroid hormone concentrations (Kuhn et al. 1998; Williamson and Davidson, 1987). As previously discussed, unpaired males with low cadmium had higher corticosterone levels, which could indicate they experience more prolonged or higher degree of stress during the mating season. This elevation of corticosterone

could contribute to the lower thyroxine levels observed in unpaired males (Fig. 2.4). Suppression of thyroxine release by corticosterone could limit the effects of mercury on thyroxine levels in unpaired birds, thus obscuring any positive correlation.

Collection date also influenced thyroxine levels in males in this study. The cause of the significant effect on thyroxine is not clear but could be associated with differences in trapping time and environmental conditions in 2004 and 2005. Thyroid function can be highly influenced by environmental factors such as temperature and food availability (McNabb, 2000). In 2004, a late thaw delayed Scaup dispersal from migratory rafts and movement onto the wetlands preferred for breeding. This delay could have provided more time for foraging or altered feeding behavior and success. Food restriction and fasting may decrease the conversion of thyroxine to triiodothyronine in birds, resulting in higher levels of circulating thyroxine (McNabb, 2000; Navidshad et al., 2006). Temperature differences between years could also have influenced thyroid hormone levels. Typically cold temperatures stimulate conversion of thyroxine to triiodothyronine while warm temperatures depress thyroid activity (McNabb, 2000). Environmental conditions were not measured in this study however, preventing any meaningful conclusions or explanation for the influences of collection date on thyroid hormone status. Alternatively, the year effect may be caused by uneven samples of paired and unpaired males in 2004 and 2005. Paired males had higher thyroxine concentrations and this could indicate differences in metabolism between paired and

unpaired male scaup. Data must be interpreted with caution because of possible year differences not accounted for by combining 2004 and 2005 data.

2.5 Conclusions

Results from this study provide information regarding levels of contaminants in Lesser Scaup on the breeding grounds. Levels of selenium shown to cause reproductive impairment in other waterfowl species appear to persist to the breeding ground in a small proportion (2.5%) of Lesser Scaup utilizing breeding grounds in the Northwest Territories. Selenium, cadmium and mercury also appear to be influencing other physiological processes in Lesser Scaup including corticosterone and thyroxine levels. Though many studies have detected alteration of hormone secretions by contaminants, few have examined the interactions of contaminants and other variables and how these interactions can modify expression of toxic effects. This study emphasizes the complex nature of biological systems and the importance of considering these interactions to accurately characterize the effects of contaminants.

Results indicate that paired and unpaired male Lesser Scaup from my sample have different responses to stress. Unpaired birds appeared to experience a higher degree of stress than paired birds, in individuals with low cadmium. These results are similar to other studies of waterfowl where unpaired birds showed higher corticosterone levels than paired birds during the mating season. This could indicate that an increase in the male-biased sex ratio is not resulting in any increased stress for paired individuals due to an increase in competitors or agonistic

encounters. Stable pair bonds, however, had already likely been formed before arrival on northern breed grounds. Assessment of agonistic interactions and stress responses in male Lesser Scaup earlier in migration, when stable pairs have not yet been formed, may give better indication of possible implications of changes to the sex ratio, including an increase in competition or changes in levels of stress experienced by males.

Table 2.1 Geometric mean, range, minimum and maximum liver selenium and mercury and kidney cadmium concentrations (µg/g dry weight) in paired and unpaired male Lesser Scaup (*Aythya affinis*) captured in the Northwest Territories, Canada, 2004-2005

Contaminant	Overall mean	**Paired** (n=19)			**Unpaired** (n=20)		
		Mean	Range	Min.-Max.	Mean	Range	Min.-Max.
Cadmium	9.2	11.6	92.82	0.78-93.6	7.4	48.51	1.67-50.18
Selenium	4.3	4.6	7.52	2.12-9.64	4.1	10.43	2.29-12.72
Mercury	1.3	1.5	3.15	0.56-3.71	1.2	1.6	0.52-2.12

Units are in µg/g dry weight

Table 2.2 Mean (±SE) serum corticosterone, glucose and thyroxine concentrations, body condition and body size for paired(n=19), unpaired (n=20) and combined (n=39) sample of male Lesser Scaup collected in the Northwest Territories , Canada, 2004-2005

	Corticosterone[a]	Glucose[a]	Thyroxine[b]	Body Size[c]	Body Condition[d]
Paired	38.7±5.4	2.0±0.06	8.0±0.78	0.32±0.19	14.46±10.03
Unpaired	36.9±5.8	1.8±0.11	4.32±0.51	-0.30±0.2	-13.74±7.02
Combined	37.8±3.9	1.9±0.06	6.12±0.55	-0.1×10^{8}±0.16	-0.0005±6.41

[a] - units are in ng/ml

[b] - units are in µg/dl

[c] - morphometric measurements (head to bill length, wing cord and tarsal length) were used to derive an index of body size using principal component analysis.

[d] - an index of body condition was calculated by using the residuals of the relationship between body size and body mass

Table 2.3 Final general linear model retained for in-depth consideration relating corticosterone residuals to pair status, collection date, tissue contaminants, body condition and body size on 39 male Lesser Scaup collected in the Northwest Territories, Canada, 2004-2005

Source[a]	Estimate[b]	Standard Error[c]	F[d]	P[e f]
pair status	32.617	11.079	8.667	0.007
log kidney cadmium	15.398	8.188	0.032	0.859
log liver selenium	-179.600	44.992	15.934	0.000
liver mercury	-58.502	19.160	9.322	0.005
body condition	1.134	0.467	5.910	0.022
body size	-8.456	8.528	0.983	0.331
pair status × log kidney cadmium	-33.251	11.917	7.785	0.010
log kidney cadmium × body condition	-0.323	0.146	4.899	0.036
log liver selenium × body condition	-1.196	0.530	5.092	0.033
log kidney cadmium × body size	-13.347	6.265	4.539	0.043
mercury × body size	10.288	4.256	5.842	0.023
log liver selenium × mercury	91.260	31.338	8.481	0.007

[a] The source of variability coming from the main effects and their interactions
[b] Values for the regression equation which explain the relationship between the dependent variable and the independent variable
[c] Standard error of the estimate used to obtain a t-value (for testing whether the variable is significantly different from 0) by dividing the parameter estimate by the standard error
[d] The ratio of the mean square for the model to the mean square for the error. The larger the F-value, the more significant the term.
[e] Non-significant (P>0.100) interactions and main effects (unless in interaction) excluded from model
[f] The probability that an individual predictor in the model does not explain a significant proportion of the variance, given the other variables in the model

Table 2.4 Final general linear model retained for in-depth consideration relating serum thyroxine concentrations to pair status, collection date, tissue contaminants, body condition and body size on 39 male Lesser Scaup collected in the Northwest Territories, Canada, 2004-2005

Source[a]	Estimate[b]	Standard Error[c]	F[d]	P[e f]
pair status	2.691	2.142	1.578	0.218
collection date	-0.183	0.070	6.775	0.014
mercury	2.644	0.685	1.724	0.198
pair status × mercury	-3.340	1.330	6.302	0.017

[a] The source of variability coming from the main effects and their interactions
[b] Values for the regression equation which explain the relationship between the dependent variable and the independent variable
[c] Standard error of the estimate used to obtain a t-value (for testing whether the variable is significantly different from 0) by dividing the parameter estimate by the standard error
[d] The ratio of the mean square for the model to the mean square for the error. The larger the F-value, the more significant the term.
[e] Non-significant (P>0.100) interactions and main effects (unless in interaction) excluded from model
[f] The probability that an individual predictor in the model does not explain a significant proportion of the variance, given the other variables in the model

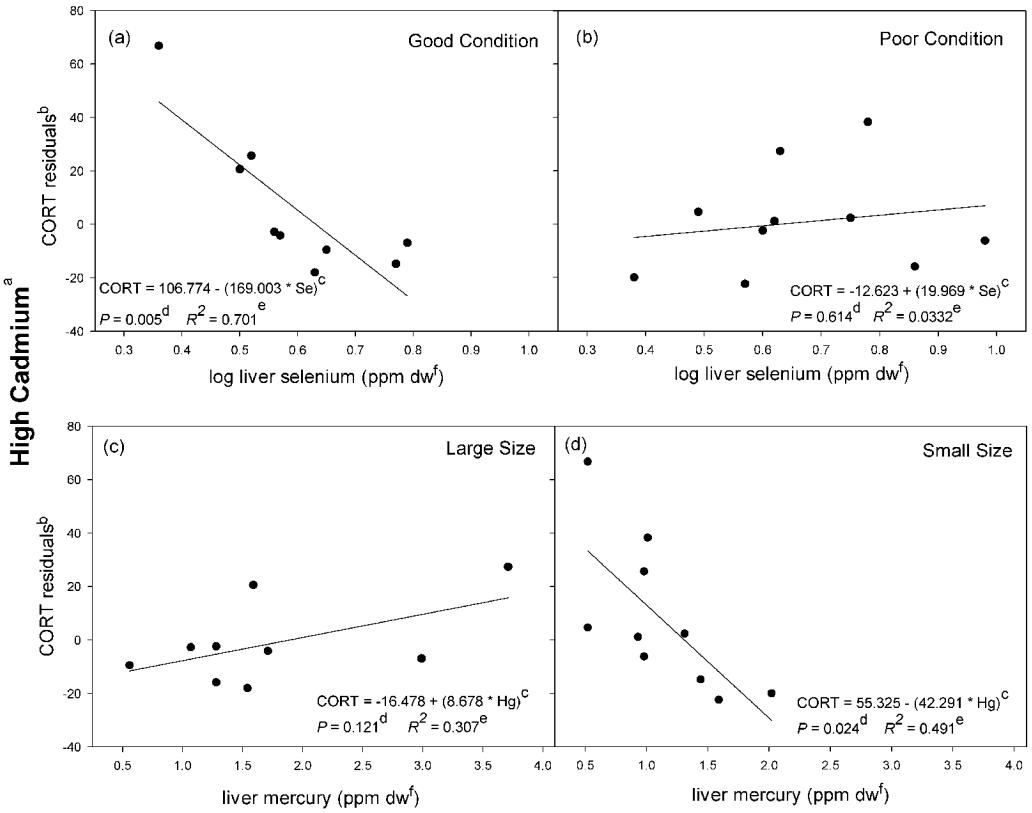

Figure 2.1. Relationships of the selenium x body condition (a,b) and mercury x body size (c,d) interactions with corticosterone (CORT) residuals in male Lesser Scaup with high (> median) kidney cadmium levels. To examine the nature of the interactions, birds were divided based on median body condition and body size before comparing corticosterone with selenium and mercury.

[a] The high cadmium group was established by separating sample based on median kidney cadmium levels and only including birds with greater than median cadmium levels

[b] Corticosterone levels were correlated to sampling time and therefore to correct for this, residuals of the relationship between corticosterone and time taken to complete sample were used

[c] Regression equation (y=b+mx) where y is the dependant variable; b is the y-intercept; m is the slope of the regression line; and x is the independent variable

[d] *P*-value indicates the probability that an observed relationship would result in a random distribution. A *P*-value of <0.05 is considered statistically significant

[e] R^2 or the coefficient of determination and is the proportion of variance in the dependent variable which can be predicted from the linear fit of the dependant to the independent variable

[f] Parts per million dry weight

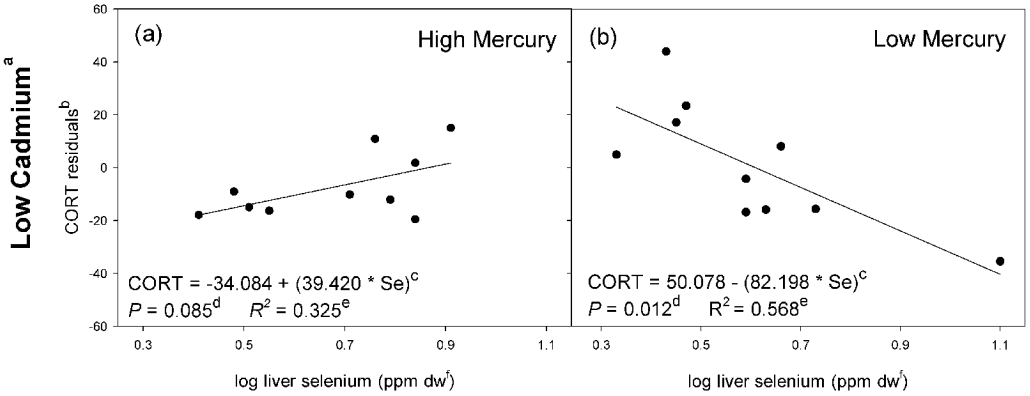

Figure 2.2 Relationship between the selenium x mercury interaction and corticosterone in male Lesser Scaup with low(< median) kidney cadmium levels. To examine the nature of the interaction, birds were divided based on median liver mercury concentrations before comparing the relationship between selenium and corticosterone in birds with a) high and b) low mercury.

[a] The low cadmium group was established by separating sample based on median kidney cadmium levels and only including birds with kidney cadmium status below median value

[b] Corticosterone levels were correlated to sampling time and therefore to correct for this, residuals of the relationship between corticosterone and time taken to complete sample were used

[c] Regression equation (y=b+mx) where y is the dependant variable; b is the y-intercept; m is the slope of the regression line; and x is the independent variable

[d] *P*-value indicates the probability that an observed relationship would result in a random distribution. A *P*-value of <0.05 is considered statistically significant

[e] R^2 or the coefficient of determination and is the proportion of variance in the dependent variable which can be predicted from the linear fit of the dependant to the independent variable

[f] Parts per million dry weight

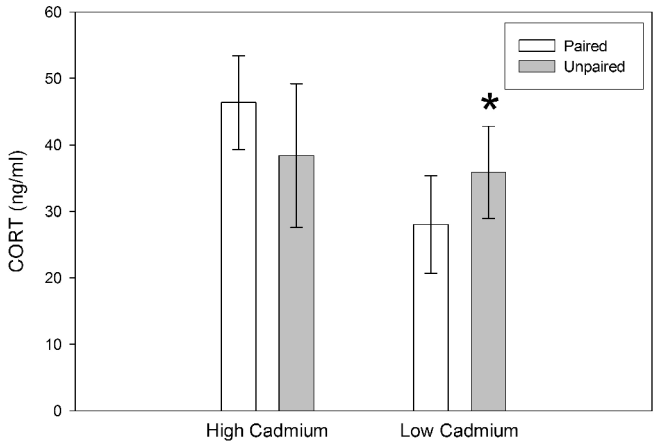

Figure 2.3 Comparison of mean (±SE) serum corticosterone levels in paired and unpaired male Lesser Scaup with high (>median) and low (≤median) kidney cadmium levels. * - indicate significantly different (GLM procedure, P<0.05) corticosterone levels in paired and unpaired individuals with low kidney cadmium

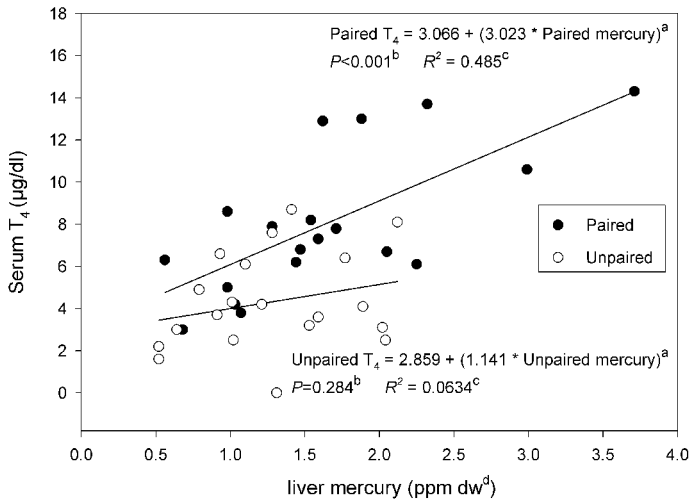

Figure 2.4 The relationship of liver mercury concentrations (ppm dry weight) and serum thyroxine (T_4)(µg/dl) in paired (n=19) and unpaired (n=20) Lesser Scaup

[a] Regression equation (y=b+mx) where y is the dependant variable; b is the y-intercept; m is the slope of the regression line; and x is the independent variable

[b] *P*-value indicates the probability that an observed relationship would result in a random distribution. A *P*-value of <0.05 is considered statistically significant

[d] R^2 or the coefficient of determination and is the proportion of variance in the dependent variable which can be predicted from the linear fit of the dependant to the independent variable

[d] Parts per million dry weight

3.0 Reproductive Readiness and Trace Element Contaminants in Male Lesser Scaup (*Aythya affinis*)

3.1 Introduction

Reproductive success relies on a complex interaction of physiological, environmental and behavioral variables. As mating season approaches and photoperiod lengthens, photoreceptors in the hypothalamus are stimulated resulting in production of gonadotropin-releasing hormone (Donham, 1979; reviewed in Farner and Wingfield, 1980; Sharp et al., 1986; Wingfield et al., 1992). Release of this hormone causes the pituitary gland to produce follicle-stimulating hormone and luteinizing hormone (Bluhm, 1992). These hormones interact with cells in the testes stimulating gonadal development, Sertoli and Leydig cell proliferation and an increase in reproductive steroid production. Testosterone is the primary steroid produced by the Leydig cells and is associated with several reproductive variables, including initiation of spermatogenesis, an increase in aggression and competitiveness, frequency of extra-pair copulation and maintenance of secondary sexual characteristics (Wingfield et al., 1990; Kirby and Froman, 2000; Dloniak and

Deviche, 2001; Davis, 2002).

Studies have examined a number of reproductive variables in an attempt to characterize their relationship with mating performance and reproductive success in birds. Testosterone concentration has been shown to play a crucial role in reproductive performance. More dominant males in social groups may have higher levels of testosterone in waterfowl species (Poisbleau et al., 2005b). Males with higher testosterone also typically win competitions with other males for mates and more efficiently defend theses mates against extra-pair copulation attempts by other males (Wingfield et al., 1990; Garamszegi et al., 2005). Individuals with high testosterone levels are more likely to attempt extra-pair copulations with neighboring females and maintain larger territories in waterfowl (Davis, 2002). Vocalization, courtship displays, plumage and other secondary sexual characteristics are often related testosterone levels and could account for female preference for males with higher testosterone (Dloniak and Deviche, 2001).

Though testosterone is the primary reproductive steroid hormone, corticosterone has also been associated with competition and mate acquisition in waterfowl. When female ducks choose a male as a mate, the chosen males has an increase in corticosterone levels, while males not chosen have no change in corticosterone (Sorenson et al., 1997). This increase of corticosterone in chosen males may be an adaptation to facilitate mobilization of energy reserves necessary for establishing dominance and defending mates. Concurrent with elevation of corticosterone in chosen males was a decrease in testosterone concentrations while

no change in hormone levels was observed in males not chosen (Sorenson et al., 1997). Reduction of testosterone could be explained by the "Challenge hypothesis", which suggests that testosterone levels increase during periods of heightened conflict such as competitions for mates and decrease once stable dominance hierarchies or territory boundaries are established (Wingfield et al., 1990). Corticosterone can also suppress testosterone production (Sapolsky et al., 2000) and this could partially account for the decrease in testosterone levels in chosen males.

An important component of reproductive success is sperm production and successful fertilization of a female. Fertilization success increases in relation to an increase in quantity of sperm transferred to the female (Moller, 1991). Quantity of sperm produced has been positively correlated to testicular size in birds (Garamszegi et al., 2005). Testes with larger mass also produce more testosterone (Garamszegi et al., 2005; Denk and Kempenaers, 2006). Testes size therefore, may affect reproductive success indirectly by influencing testosterone-dependent behavior related to mate competition and guarding, and directly by affecting the quantity of sperm produced and delivered. Histological examination of testes has been used to characterize changes during reproductive development and assess testes function (Mather and Wilson, 1964; Amann, 1986). Of variables measured, seminiferous tubule diameter was considered to be one of the more sensitive indicators of testicular function and contaminant-related effects on testes structure (Amann, 1986; Young et al., 2001).

Like most birds, the left testis is typically larger than the right in Lesser Scaup (Anderson and Warner, 1969). This asymmetry can be quite variable, with testes occasionally being equal in size or even with right testis being larger. It has been proposed that the right testis may develop to compensate for problems or dysfunction with the left testis or as a result of compromised body condition, thus decreasing the degree of testicular asymmetry (Moller, 1994). Expression of testicular asymmetry may also be related to age in birds, with older males showing a greater degree of testicular asymmetry than young males (Birkhead et al., 1997; Graves, 2004). Secondary sexual characteristics such as tail length and plumage have been correlated to the degree of testicular asymmetry in male barn swallows (*Hirundo rustica*) and house sparrows (*Passer domesticus*) (Moller, 1994). Other studies, however, have shown no relationship between sexual characteristics and testicular asymmetry (Kimball et al., 1997; Birkhead et al., 1997; Birkhead et al., 1998).

Male reproductive physiology and behavior can be altered by environmental contaminants, including trace elements and metals. The testicular toxicity of cadmium has been well documented. A decrease in testosterone production has been observed in Leydig cell cultures in response to cadmium (Laskey and Phelps, 1991; Ng and Lui, 1990). Cadmium can reduce testes weight, testosterone, sperm production and cause histological alterations in mammals (Laskey et al., 1984; Lymberopoulos et al., 2003). A decrease in testicular development and growth, alteration of testicular morphology and suppression of spermatogenesis have also

been associated with exposure to cadmium in birds (Richardson et al., 1974; White et al., 1978; Hughes et al., 2000; Swiergosz and Kowalska, 2000). Mercury can decrease Leydig cell viability and suppress testosterone production in cell cultures (Ng and Lui, 1990). Mercury also decreased testosterone levels and number of sperm in rat testes (Friedmann et al., 1998). Selenium levels can influence steroid hormone concentrations (Freeman and Sangalang, 1977; Wayland et al., 2002) and alter seminiferous tubule diameter and morphology (Behne et al., 1996; Green and Albers, 1997; Zhang et al., 2004).

The primary objective of this section was to examine and characterize how a variety of reproductive variables relate to pair status in male Lesser Scaup. Testosterone and corticosterone levels have been linked to aggression and could influence the outcome of competition for mates. Testosterone levels have also been linked to the expression of secondary sexual characteristics and courtship behaviors which could influence mate preference and selection by females. As asymmetry in size between left and right testes has also been linked to the expression of secondary sexual characteristics, it will be considered as a possible influence on pairing success. Testicular size will be considered as larger testes may produce more testosterone and could therefore indirectly affect level of aggression, expression of secondary sexual characteristics and the ability to attract a mate. In addition, structural size and body condition were considered as general indicators of male quality and included to assess possible influences on pair status.

The second objective was to consider the possible effects of contaminants

and body condition on testosterone, testes mass and seminiferous tubule diameter. As in Chapter 2, interactions of trace elements will be considered when assessing influences of contaminants on the dependent reproductive variables. Trace elements have been shown to alter steroid secretions and could be affecting testosterone levels in male Lesser Scaup. In addition, corticosterone suppresses gonadal steroids and will also be included to test for effects on testosterone levels.

3.2 Materials and Methods

3.2.1 Sample Collection and Processing

Tissue and blood samples from paired and unpaired Lesser scaup males used in the previous chapter were used for analysis in this section. Blood samples were collected immediately following capture of males via jugular venipuncture, placed in serum separator tubes and centrifuged. Time of day and time taken to complete blood sampling were recorded following sample collection. Males of known pair status were sacrificed, weighed to the nearest 10 g and morphometric measurements (to nearest 0.1mm) (head to bill, wing cord and tarsal lengths) were recorded and an index of body size was calculated using principal component analysis. Body mass was regressed against PC1 scores to correct for body size and residuals from this relationship were used as an index of body condition. Liver and kidneys were removed and processed as in Chapter 2. Fresh liver samples were submitted to the University of Saskatchewan Prairie Diagnostic Service Toxicology lab (Western College of Veterinary Medicine, Saskatoon, SK) for selenium and total mercury residue analysis. Freeze dried kidneys were analyzed for cadmium

concentrations at the University of Saskatchewan Toxicology Centre (Saskatoon, SK) (Berney et al., 2003). Selenium was measured using an inductively coupled plasma-emission spectrophotometer equipped with a hydride generator accessory (Thermo Jarrel Ash Corp, Franklin, MA, USA) (Hoffman et al., 1998). Total mercury was measured using an inductively coupled plasma-mass spectrophotometer (Thermoelectron Corp., Chesire, United Kingdom)(Shuqin et al., 1999). Cadmium was measured using a Varian SpectrAA 220Z graphite furnace equipped with a graphite tub atomizer (GTA-110Z).

3.2.2 Testes Measurement and Histological Examination

Right and left testes were removed from the carcass, and weighed to the nearest 0.1 gram using a digital Mettler scale. Maximum length and width of each testis was measured to the nearest 0.1 mm using digital calipers (Mitutoya Corporation, USA). The volume of each testis was estimated using the formula for a prolate spheroid: testis volume (mm^3) = $4/3\pi a^2 b$ where a is the radius of the width and b is the radius of the length (Moller, 1994). Volume was used to calculate the asymmetry between left and right testes. Relative asymmetry, used to adjust for testes size, was calculated using the formula: relative asymmetry = left volume-right volume/0.5(left volume + right volume) (Birkhead et al., 1998).

Following measurement, the testes were placed in 10% neutral buffered formalin (BDH Diagnostics, Dorset, U.K.) for a minimum of two weeks. The left testis was embedded in paraffin and sectioned at 5 μm at two different randomly selected levels within the testis. The sections were adhered to microscope slides

and stained with hematoxylin and eosin (Sigma-Aldrich Canada Ltd.). The sections were examined by an observer blind to treatment using an Olympus CX-41 compound microscope (Olympus America Inc., New York) The diameter of the minor axis of 10 seminiferous tubules was measured using an ocular micrometer in each of the two tissue sections. Only tubules with a major axis less than the minor axis + 10 μm were selected to ensure only cross sections were measured. The average diameter of the tubules measured was used as an indication of testicular condition (Young et al., 2001).

3.2.3 Hormone Analysis

Hormone analysis was conducted by the University of Saskatchewan Prairie Diagnostic Service Endocrinology lab at the Western College of Veterinary Medicine using standard operating procedures. Total serum testosterone concentrations were measured using a solid phase testosterone radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA, USA) (Deviche et al., 2001). Manufacturer's protocols were followed for the assay except that 50μl of sample were used instead of the recommended 25μl. Intra-assay coefficient of variation was 4.6, 5.0 and 6.2% with means of 1.30, 4.83 and 6.20μl respectively. Serum corticosterone concentrations were measured using a corticosterone ^{125}I radioimmunoassay (RIA) kit (MP Biomedicals, LLC, Orangeburg, NY, USA) (Sorenson et al., 1997). Manufacturer's protocol was followed except that serum samples were diluted 1:100 rather than 1:200. Intra-assay coefficients of variation were 6.4% and 3.3% with means of 76.9 and 522.6 ng/ml respectively.

3.2.4 Statistics

All data were analyzed using SPSS 14.0 (SPSS Inc. 2005). Morphometric measurements were used to derive an index of body size using principal component analysis. The first axis of the principal component analysis (PC1) explained 49.4% (λ_1=1.48) of variability in structural size. The component loading of morphometric measurements for PC1 were wingchord (-0.002), tarsus length (0.581), and head to bill length (0.581). Body mass was regressed against PC1 scores and residuals from this relationship were used as an index of body condition. Although low sample size of paired individuals in 2004 prevented comparison of year, few difference between year, with the exception of body size, were detected between unpaired birds from 2004 and 2005. Data from the two years was therefore lumped together.

Prior to further analysis, all data were evaluated for normality using a Kolmogorov-Smirnov test and where necessary, were log-transformed to normalize data. Tests for normality showed non-normal distributions for liver selenium, kidney cadmium and serum testosterone so concentrations were therefore $\log_{10}$-transformed prior to further analysis. As in the previous chapter, corticosterone levels were highly correlated to handling time so residuals of the relationship were used to correct for handling time.

The relationships of corticosterone residuals, testosterone, testicular asymmetry, body size, body condition and testicular mass to pair status were examined using a binary logistic regression. An enter procedure was used with a

significance of $P \leq 0.100$ value required for inclusion of a term in the model. In addition, general linear models (GLM) were used to examine the effects of body condition, body size and contaminants on several reproductive variables including testosterone levels, testes mass and seminiferous tubule diameter. Analyses were limited to two-way, *a priori* defined interactions. Modeling procedure followed that described in the previous chapter and by Alisauskas and Ankney (1994), where non-significant terms ($P>0.10$) were systematically removed from significant models ($P<0.05$).

3.3 Results

3.3.1 Trace Element and Contaminant Levels

As reported in Chapter 2, the geometric mean concentration of cadmium was 9 µg/g dry weight with a minimum of 0.78 µg/g and a maximum of 93.6 µg/g. Selenium concentration ranged from 2.12 to 12.72 µg/g dry weight with a mean of 4.33 µg/g. Mercury concentrations ranged from 0.52 to 3.71 µg/g dry weight with a mean of 1.31 µg/g dry weight (Table 2.1).

3.3.2 Pair Status

Examination of effects of corticosterone residuals, testosterone, testicular mass, testicular asymmetry, body condition and body size produced a significant model ($\chi^2 = 9.997$, df=2, $P=0.006$). Paired birds were in significantly better body condition than unpaired birds (score=4.965; df=1; $P=0.026$) (Fig. 3.1). Paired birds also had larger body size than unpaired birds (score=3.808; df=1; $P=0.051$) (Fig. 3.2).

3.3.3 Testosterone (Table 3.1)

The effects of cadmium, mercury, selenium, body condition, body size and corticosterone on testosterone levels in male Lesser Scaup were considered. The resultant model however, was not significant ($F_{10,28}$=0.307, P=0.989).

3.3.4 Testes Mass (Table 3.1)

The effects of cadmium, mercury, selenium, body condition and size and testosterone on testes mass were considered. The resultant model examining the effects of these variables was not significant. ($F_{9,29}$=0.455, P=0.897).

3.3.5 Tubule diameter

The effects of body condition and size, left testicular mass, cadmium, selenium and mercury on seminiferous tubule diameter was examined resulting in a significant model ($F_{2,36}$=17.558, P<0.001). There was a highly positive correlation between the mass of the left testes and seminiferous tubule diameter (R^2=0.397, n=39, P<0.0001)(Fig. 3.3). There was also a significant negative correlation between liver selenium levels and seminiferous tubule diameter (R^2=0.123, n=39, P=0.009)(Fig. 3.4).

3.4 Discussion

3.4.1 Trace Elements and Contaminants

In this study, 21 of 39 (54%) of ducks had kidney cadmium concentrations above levels considered background for waterfowl (7 µg/g dry weight) (Puls, 1994), though none were above the 100 µg/g dry weight threshold for major toxic effects (Furness, 1996). Cadmium can cause sublethal changes in physiology

including testicular damage, including reduced testicular weight, suppressed testosterone secretions and sperm production and histological alterations (Laskey et al., 1984; Lymberopoulos et al., 2003). Kidney cadmium concentrations of approximately 50 μg/g wet weight (~175 μg/g dry weight) have been associated with alteration of testes function and histology in Mallard ducks (White et al., 1978). Liver cadmium residues as low as 44 μg/g wet weight were associated with decreased testes weight and spermatogenesis in Japanese quail (Richardson et al., 1974). Compared to these studies, concentrations of cadmium in my sample were well below levels shown to result in changes in testicular function or morphology.

Lesser Scaup males had liver selenium concentration ranging from 2.12 to 12.72 μg/g dry weight with only one being above 10 μg/g dry weight, which is the level shown to have caused reproductive impairment in females (Heinz, 1996). Selenium may also cause reproductive damage in males including reduced testes size and seminiferous tubule diameter in Mallard ducks (Green and Albers, 1997). Unfortunately in previous studies, tissue selenium concentrations were not correlated to these changes in testicular morphology. Mercury concentrations in all Lesser Scaup were below threshold for toxic effects in birds (15 μg/g dry weight) (Zillioux et al., 1993).

3.4.2 Pair Status

In the present study, body condition was an important indicator of pair status. Other studies have observed a relationship between social status and body condition in waterfowl (Kotrschal et al., 1993; Poisbleau et al., 2006) and other bird

species (i.e., songbirds, chickens, etc.) (Carrascal et al., 1998; Gosler and Carruthers, 1999; Cloutier and Newberry, 2000; Jenkins et al., 2001; Parker and Ligon, 2002). More dominant individuals typically have better body condition than subordinate individuals. Better body condition may be a reflection of a greater access to food resources or the ability to defend food resources from subordinates (Jenkins et al., 2001; Kotrschal et al., 1993). Body condition could indicate higher muscle mass providing the strength to win physical encounters involved in mate competition or guarding (Gosler and Carruthers, 1999) or greater fat reserves providing energy required for mating or physical competition and for adding to protein reserves lost during migration (Gauthier et al., 1992; Jenkins et al., 2001; Poisbleau et al., 2006). In my sample, body condition was calculated using total body mass, so relative contribution of muscle and fat mass to body condition is not known. Body condition has also been positively correlated to the expression of secondary sexual characteristics such as male ornamentations (Buccholz, 1997, Parker and Ligon, 2002) and plumage (Keyser and Hill, 1999; 2000; Doucet, 2002). This could indicate that better body condition provides for nutritional or energetic requirements for development and maintenance of secondary sexual characteristics (Parker and Ligon, 2002).

Body size was related to pair status in this study, with paired birds being larger than unpaired birds. This supports the suggestion that large-sized birds have an advantage in physical competitions and encounters with smaller birds (Poisbleau et al., 2006) contributing to the ability of larger males to acquire mates. Larger,

more dominant birds may also outcompete smaller individuals for food resources or areas of higher food availability, contributing to the development of better body condition (Carrascal et al., 1998). Poor nutrition during development has been shown to result in smaller body size and altered behavior, such as vocalization in adult songbirds (Soma et al., 2006), and could partially account for the influences of body size in my sample. Data must be interpreted cautiously, however, because of possible year difference which are not accounted for by combining the data

Though not important contributors to pair status in this study, several other factors are known to influence mate acquisition and pair status. Møller (1994) found a significant relationship between the degree of testicular asymmetry and secondary sexual characteristics in barn swallows (*Hirundo rustica*) and house sparrows (*Passer domesticus*) and suggested that males with a greater degree of asymmetry had a selective advantage. Several other studies, however, found no correlation between the degree of asymmetry and sexual characteristics or behaviors (Birkhead et al., 1997; 1998; Kimball et al., 1997). In this study, testicular asymmetry was not related to pair status. At the time of collection, however, stable pair bonds had already been formed. Any influence of testicular asymmetry on secondary sexual characteristics and indicators of male quality may only have been visible earlier in the mating season during competition for mates.

Testosterone influences aggression and expression of secondary sexual characteristics, contributing to pairing and mating success (Wingfield et al., 1990; Garamszegi et al., 2005). Testosterone levels are dynamic however, and may

decrease once stable dominance relationships or pair bonds have been formed (Sorenson et al., 1997). Though Lesser Scaup are one of the latest duck species to pair seasonally, nearly all pairs are formed by late April and early May (Austin et al., 1998). Individuals in my sample had likely been paired for several weeks or months before being captured. Testosterone levels in paired males, therefore, may have already declined following pair formation. Assessment of levels earlier in migration may detect differences in testosterone levels between individuals that pair successfully and those that remain unpaired.

Larger testes have been associated with higher concentrations of testosterone and could, therefore, indirectly influence mating and reproductive variables, and the ability to acquire mates (Garamszegi et al., 2005; Denk and Kempenaers, 2006). Testicular mass was not related to pair status in my sample. As has been suggested for testosterone and testicular asymmetry, this could be because sampling occurred when stable pair bonds had formed instead of during peak periods of competition for mates

Some studies of waterfowl have observed a relationship between pair or social status and corticosterone (Sorenson et al., 1997; Poisbleau et al., 2005a). Corticosterone levels in paired males ducks may increase following successful pairing with females, possibly as an adaptation to mobilize energy reserves to help defend a newly acquired mate (Sorenson et al., 1997). These differences in corticosterone levels were typically observed a few days after the mate selection process. Pair status in my sample however, was not related to corticosterone

levels. Since pairs had likely been formed for several weeks before they were captured, differences in corticosterone levels may no longer be visible. Pair status and corticosterone were related in analysis of data in chapter 2 however. These differences were only seen when interactions with cadmium levels were considered. Cadmium was not considered for influences on pair status in analysis for this chapter and could account for the absence of any observed relationship between pair status and corticosterone.

3.4.3 Testosterone

Testosterone was not influenced by contaminants, body condition and size or corticosterone. Other studies have observed altered testosterone levels in response to a variety of physiological variables and environmental factors. Feed restriction and decreased body condition resulted in lower testosterone levels in male Red-legged partridges (*Alectoris rufa*) (Perez-Rodriguez et al., 2006). Environmental contaminants including cadmium and mercury can alter testicular function and suppress production and secretion of testosterone (Sangalang and O'Halloran, 1972; Ng and Liu, 1990; Laskey and Phelps, 1991; Friedmann et al., 1998). Cadmium concentrations in my sample were less than those previously shown to cause altered testicular function or morphology in birds (Richardson et al., 1974; White et al., 1978), and this might account for the lack of influence on testosterone levels. Mercury levels were comparable to levels considered background values in birds (Scheuhammer, 1987) and were likely also too low to cause a suppression of testosterone secretions. Corticosterone can suppress

gonadal secretions of testosterone (Silverin, 1998; Sapolsky et al., 2000). Since testosterone levels may decline when stable pair bonds have been formed however, testosterone levels in the scaup may have already declined and thus, obscured any suppression of testosterone by corticosterone or environmental contaminants. Measurement of testosterone levels earlier in migration, prior to pairing may clarify whether contaminants or other variables are affecting testosterone levels in male Lesser Scaup.

3.4.4 Testes mass

Testicular mass was not influenced by contaminants, body condition or body size. Other studies have noted that testicular mass can be influenced by a variety of variables. Testicular size is positively correlated to body condition in small mammals (Schulte-Hostedde et al., 2005) and testes mass was positively associated with body mass in a survey of 247 bird species (Møller, 1991). Several studies have observed a decrease in testes mass associated with cadmium toxicity (Richardson et al., 1974; White et al., 1978; Laskey et al., 1984; Lymberopoulos et al., 2003). Suppression of testicular growth and size have typically been associated with at concentrations higher than those in birds from my sample and this might account for the absence of any observed influence by contaminants. Other studies have found that testicular mass is not as sensitive an indicator of testicular toxicity as histological variables including seminiferous tubule diameter (Amann, 1986).

3.4.5 Seminiferous Tubule Diameter

Results from this study indicate that seminiferous tubule diameter can be

influenced by testicular mass and selenium levels. There was a significant positive correlation between testicular mass and tubule diameter indicating that larger testes had larger seminiferous tubules (Fig. 3.3). Selenium levels, had a negative relationship with tubule diameter (Fig. 3.4). Other studies also detected a relationship between selenium and seminiferous tubule diameter. Rats fed a selenium deficient diet had a variety of reproductive effects including decreased testosterone production and a reduction in seminiferous tubule diameter (Behne et al., 1996). Elevated concentrations of selenium can also affect testicular morphology. Mallard ducks fed diets containing elevated concentrations of selenium had reduction in testicular mass, fewer sperm cells within seminiferous tubules, and a decrease in tubule diameter (Green and Albers, 1997). Increasing concentrations of dietary selenium also caused changes to testes and seminiferous tubule structure in rats (Zhang et al., 2004). Though mechanisms of testicular alteration by selenium are poorly understood, they may be associated with altered testosterone synthesis or concentration of selenium containing proteins in gonadal tissue (Behne et al., 1996).

3.5 Conclusions

In this study, large-sized birds of better body condition appeared to be more likely to acquire and defend mates. Hormonal variables including testosterone and corticosterone, that have been shown to influence aggression and the ability to outcompete other males for females, had no apparent affect on paired status of male Lesser Scaup, when these parameters were measured. Sorenson et al. (1997) found

that hormone levels in waterfowl are dynamic, and can be influenced by social stimuli such as pairing and selection of males by females. Once paired, levels of testosterone can decline while corticosterone concentrations may increase. Stable pair bonds had likely already been formed in Lesser Scaup upon arrival at breeding grounds in the Northwest Territories. To more reliably measure the relative effect of hormone levels on ability of male Lesser Scaup to successfully acquire and defend a mate, it is likely necessary to measure these variables earlier in migration, prior to pair bond formation. Timing of sampling could also have limited my ability to detect effects of contaminants on testosterone levels. Collection of samples during periods of peak testosterone secretion could provide a more accurate assessment of whether contaminants have any influence on testosterone in male Lesser Scaup.

Cadmium and mercury can affect testicular morphology, growth and development. In my sample, however, contaminants had no influence on testes mass or histology. Concentrations observed were below tissue residues previously reported to affect testicular variables in birds (Richardson et al., 1974; White et al., 1978) and this could account for the lack of observed affect. Selenium concentrations had a negative correlation with seminiferous tubule diameter. Other studies have observed that both selenium deficiency (Behne et al., 1996) and elevated concentrations can cause a reduction of seminiferous tubule diameter (Green and Albers, 1997; Zhang et al., 2004). Selenium is an essential element for animal nutrition, but results from this and other studies could be an example of the

narrow therapeutic range between required and toxic levels of this element.

Table 3.1 Mean (±SE) serum testosterone, testicular mass and volume, relative asymmetry and seminiferous tubule diameter for paired(n=19), unpaired (n=20) and total (n=39) sample of male Lesser Scaup collected in the Northwest Territories , Canada

	Testosterone[a]	Testicular Mass[b]			Testicular Volume[c]			Relative asymmetry[d]	Tubule diameter[e]
		Left	Right	Combined	Left	Right	Combined		
Paired	1.1±0.3	2.4±0.2	2.2±0.2	4.5±0.4	2999±256	2836±227	5836±469	0.05±0.04	160.3±4.3
Unpaired	1.3±0.2	2.3±0.2	2.2±0.2	4.6±0.3	2695±208	2777±216	5472±361	-0.03±0.08	158.9±4.6
Total	1.2±0.2	2.3±0.1	2.2±0.1	4.5±0.2	2843±164	2806±155	5649±291	0.01±0.05	159.6±3.1

[a] units in ng/ml
[b] units in grams
[c] calculated using formula testis volume (mm^3) = $4/3\pi a^2 b$ where a is the radius of the width and b is the radius of the length (Møller, 1994)(units in mm^3)
[d] calculated using the formula relative asymmetry = left volume-right volume/0.5(left volume + right volume) (Birkhead et al., 1998)
[e] units in µm

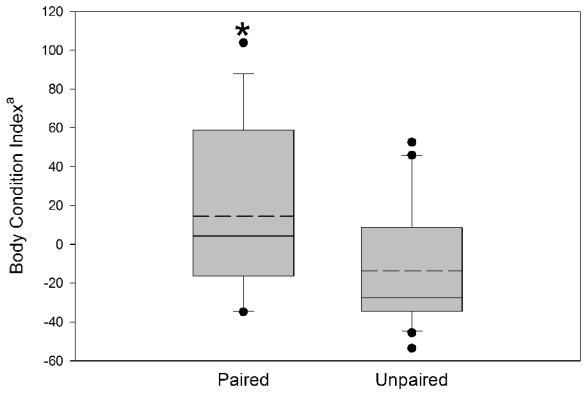

Figure 3.1 Body condition and pair status in male Lesser Scaup captured in the Northwest Territories. Mean (--), median (-) and outliers (•) are reported. Vertical lines extend to 5th and 95th percentiles. ∗ - indicates significant difference (Logistic regression, *P*=0.02) between paired and unpaired male body condition.

[a] - an index of body condition was calculated by using the residuals of the relationship between body size and body mass

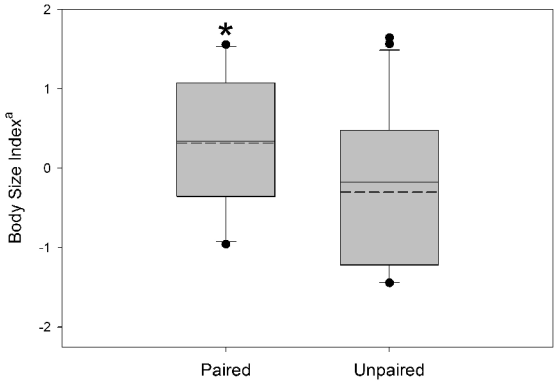

Figure 3.2 Comparison of body size for paired and unpaired male Lesser Scaup captured in the Northwest Territories. Mean (--), median (-) and outliers (•) are reported. Vertical lines extend to 5th and 95th percentiles. ∗ - indicates significant difference (Logistic regression, *P*=0.05) between paired and unpaired male body size.

[a] - morphometric measurements (head to bill length, wing cord and tarsal length) were used to derive an index of body size using principal component analysis.

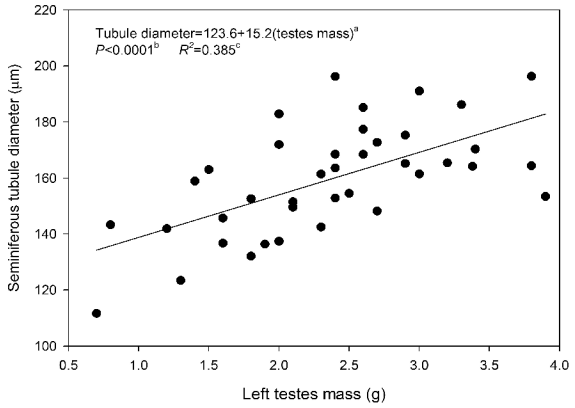

Figure 3.3 Relationship between left testes mass and the diameter of the minor axis of seminiferous tubules in left testes. Only tubules with a major axis diameter less than the minor axis + 10 µm were used to ensure tubes measured were cross sections.

[a] Regression equation (y=b+mx) where y is the dependant variable; b is the y-intercept; m is the slope of the regression line; and x is the independent variable

[b] *P*-value indicates the probability that an observed relationship would result in a random distribution. A *P*-value of <0.05 is considered statistically significant

[c] R^2 or the coefficient of determination and is the proportion of variance in the dependent variable which can be predicted from the linear fit of the dependant to the independent variable

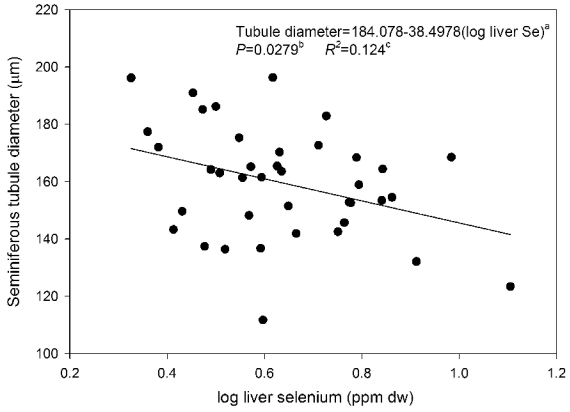

Figure 3.4 Relationship between liver selenium and seminiferous tubule diameter in left testes. Only tubules with a major axis diameter less than the minor axis + 10 µm were used to ensure tubes measured were cross sections.

[a] Regression equation (y=b+mx) where y is the dependant variable; b is the y-intercept; m is the slope of the regression line; and x is the independent variable

[b] *P*-value indicates the probability that an observed relationship would result in a random distribution. A *P*-value of <0.05 is considered statistically significant

[c] R^2 or the coefficient of determination and is the proportion of variance in the dependent variable which can be predicted from the linear fit of the dependant to the independent variable

[d] parts per million dry weight

4.0 Effects of L-Selenomethionine on Stress Response and Behavior in Captive Male Lesser Scaup (*Aythya affinis*)

4.1 Introduction

Corticosterone is considered one of the hallmark characteristics of the avian stress response, and its release leads to mobilization of energy reserves, stimulation of the immune system and altered behavior (Siegel, 1980; Silverin, 1998; Sapolsky et al., 2000). The corticosterone response is a general response to stress, however, and can be stimulated by a variety of environmental (e.g., storms, drought, breeding latitude) and life history (e.g., presence of predators, human disturbance) factors (Silverin, 1998; O'Reilly and Wingfield, 2001). For example, both basal and stress-induced levels of corticosterone show significant daily and seasonal variation in several bird species (Silverin, 1998; Remage-Healey and Romero, 2001; Landys et al., 2004). There is considerable evidence that individuals at different positions within a social hierarchy respond differently to stressors (Kotrschal et al., 1998; Senar et al., 2000; Poisbleau et al., 2005b). Significant variability can be introduced by trapping and capturing activities and the amount of time left in traps (Romero

and Romero, 2002).

Contaminants can affect corticosterone levels by altering adrenal structure or by modifying activity of the hypothalamus-pituitary-adrenal (HPA) axis (Richardson et al., 1974; Di Giulio and Scanlon, 1984b; Colby et al., 1997; Capen, 2001; Wayland et al., 2002; Mayne et al., 2004). This includes selenium, which has been negatively correlated with corticosterone levels (Freeman and Sangalang, 1977; Wayland et al., 2002). As seen in chapter 2, interpreting and characterizing influences of selenium on corticosterone levels can be complicated by interactions with contaminants such as mercury and other variables including body condition.

Elevated concentrations of selenium have been observed in Lesser Scaup and it has become one of the primary contaminants of concern in continental scaup populations (Custer and Custer, 2000; Custer et al., 2000; Custer et al. 2003; Petrie, 2004). Elevated levels have also been observed in zebra mussels, a non-native species that has become a preferred food source for diving ducks wintering or staging on the Great lakes (Petrie and Schummer, 2002). Mean levels of selenium found in spring zebra mussel tissue were 10.8 ppm (Petrie, 2004). Lesser Scaup are thought to accumulate elevated selenium concentrations by feeding on Zebra Mussels while staging on the Lower Great Lakes during spring migration. It is currently thought that selenomethionine is the most likely form of selenium transferred up the food chain to higher organisms such as Lesser Scaup (Fan et al., 2005).

In this study, the effects of selenium (in the form of selenomethionine) on

corticosterone levels in captive Lesser Scaup were investigated. Male Lesser Scaup were fed diets containing different levels of selenium and effects on stress response were assessed. Since a stress induced rise in corticosterone can also stimulate behavioral changes, behavior patterns of the different treatment groups were also considered. Using captive individuals provided a controlled environment limiting the variability introduced to corticosterone levels by external factors such as environmental influences, differences in life history or other contaminants.

4.2 Materials and Methods

This study used 18 captive male Lesser Scaup housed in a mixed flock with females in the Western College of Veterinary Medicine at the University of Saskatchewan. These males were initially collected from eggs throughout various locations in Saskatchewan and Manitoba and reared indoors in the Animal Care Unit. Birds were fed a commercially prepared duck and goose grower (Co-op Feeds, Saskatoon, Sk.) *ad libitum* and were provided with swimming pool and water at all times. Males were randomly selected and divided into three dose groups of six birds each. Dose groups were moved to separate compartments and each received a diet containing a different concentration of selenium *ad libitum* and were provided with fresh water and a pool for swimming. Light conditions within the Animal Care Unit were regularly changed to mimic naturally-occurring photoperiod.

4.2.1 Diet Preparation

Diets were prepared in the Department of Animal Sciences at the University

of Saskatchewan. Diet consisted of 75.25% ground wheat, 10% canola meal, 9.3% soybean meal, 2% canola oil and 3.47% commercially produced micronutrient mixture (Nutrimax Inc., Greensboro, N.C.). Dose groups of 7.5 ppm and 15 ppm were designed to represent environmentally relevant selenium concentrations (mean 10.8 ppm) found in zebra mussels from the Great lakes (Petrie, 2004). The control diet contained only nutritionally required levels of selenium (sodium selenite) found in micronutrient mixture, equaling approximately 0.3 ppm or 7.5 mg/25 kg of diet. L-selenomethionine (TCI America, Portland, Or.) was added to the diet mixture to develop treatment groups. For the low dose group, 180 mg of selenomethionine was added to 25 kg of diet mixture resulting in total calculated selenium (selenomethionine plus sodium selenite from supplement) levels of 7.5 ppm. For the high dose diet, 367.5 mg of selenomethionine was added to 25 kg of diet resulting in total levels of 15 ppm.

4.2.2 Stress Analysis and Sample Collection

Birds were fed experimental diets for 40 days (meant to mimic duration of stay of Scaup staging on the Great Lakes), and then underwent blood collection to analyze corticosterone response to stress and blood selenium levels. Following this dosing period, one male from each group was randomly selected to assess their stress response. Since entrance into enclosures would stress all individuals in the group, blood was collected from one male per group each day until all birds had been sampled. Since corticosterone secretions can be influenced by circadian rhythms (Remage-Healy and Romero, 2001), blood sampling occurred at

approximately the same time each morning to eliminate any effects of time of day. Baseline corticosterone levels may not entirely reflect an individual's response to stress in birds (Silverin, 1998). To more completely characterize stress response in males, blood samples were collected at intervals (0, 15, 30 and 60 minutes) over a one hour period.

Upon entering the enclosure of each dose group, a male was randomly selected and approximately 1.5 ml of blood was collected via jugular venipuncture. Following completion of sampling, the interval from entering the enclosure until blood sampling was recorded. The blood sample was divided with 1 ml of sample was placed in a serum separator vacutainer for corticosterone analysis, and 0.5 ml of sample was placed in a heparinized vacutainer for measurement of whole blood selenium levels. Following collection of the first sample, the male was placed in a darkened box and then removed at 15, 30 and 60 minutes after completion of the first blood sample, for collection of additional samples. At each of these intervals, 1 ml of blood was collected and placed in serum separator vacutainers, and the time taken to complete blood sample collection was recorded. Vacutainers were stored in a cooler with ice packs until being centrifuged at 3000 rpm for 10 minutes. Tubes were then frozen at -20°C until analysis could be done.

4.2.3 Corticosterone and Selenium Analysis

Corticosterone analysis was conducted by the University of Saskatchewan Prairie Diagnostic Service Endocrinology lab at the Western College of Veterinary Medicine, Saskatoon, SK using standard operating procedures. Serum

corticosterone concentrations were measured using a corticosterone ^{125}I radioimmunoassay (RIA) kit (MP Biomedicals, LLC, Orangeburg, NY, USA) (Sorenson et al., 1997). Manufacturer's protocol was followed except that serum samples were diluted 1:100 rather than 1:200. Intra-assay coefficients of variation were 3.5% and 1.4% with means of 68.1 and 440.9 ng/ml respectively.

Whole blood samples were submitted to the University of Saskatchewan Prairie Diagnostic Service Toxicology lab (Western College of Veterinary Medicine, Saskatoon, SK) for selenium analysis. Blood samples were digested in 69-70% nitric acid using a microwave accelerator reactor system (CEM Corp., Matthews, NC, USA). Samples were then analyzed using an inductively coupled plasma-mass spectrophotometer (Thermoelectron Corp., Chesire, United Kingdom).

4.2.4 Behavioral Analysis

Prior to beginning observations, a male (not included in a dose group) and a female were separated and housed in the observation room to acclimate for one week. After completion of blood sampling procedures, males were randomly introduced into the observation room with the resident pair. After acclimating to the room for 15 minutes, interactions between the introduced male and pair were recorded using closed-caption television equipment for 30 minutes. Videos were analyzed by an observer blind to treatments using Etholog behavioral analysis software (Ottoni, 2000). Distance of introduced male from pair (recorded each minute) was estimated based on number of duck lengths (female length) between

individuals. Instances of maintenance behavior (preening, head shaking, feeding/drinking) and number of agonistic interactions with male and female were recorded whenever they were observed.

4.2.5 Statistics

All data were analyzed using SPSS 14.0 (SPSS Inc. 2005). All data passed tests for normality (Kolmogorov-Smirnov, $P>0.05$) so no data transformation was required. Corticosterone concentrations were not correlated to time taken to complete blood samples and therefore use of residuals to correct for handling time was not required. Blood selenium levels for the three groups were compared using a one-way analysis of variance (ANOVA) with a *post hoc* Tukey's test for pairwise comparison of the treatment groups when significant differences were detected. Multivariate analysis of variance (MANOVA) was used to simultaneously compare the four corticosterone samples representing stress response of the three treatment groups. Multivariate analysis of variance was also used to assess differences in a variety of behaviors among treatment groups.

4.3 Results

Several males died early in this study. One male in the control group died from starvation due to a refusal of the newly introduced diet which was in mash form as opposed to standard diet which was in pellet form. One male from the 15 ppm dose group died from an intestinal impaction after consuming a large pin feather that became lodged in the jejunum. Another male from the 15 ppm treatment group died from hepatic amyloidosis. This left sample size of n=5

(control), n=6 (7.5 ppm) and n=4 (15 ppm). Finally, prior to the onset of behavioral observation, another male from the 15 ppm treatment group damaged a wing which became swollen and that male was therefore not included in the behavior portion of the study.

4.3.1 Blood Selenium

Mean blood selenium levels for control, 7.5 ppm and 15 ppm treatment groups were 0.19, 0.74 and 1.01 ppm, respectively (Fig. 4.1). Analysis of whole blood selenium indicated that levels were significantly different among treatment groups ($F_{2,12}$=155.139; P<0.001). Selenium levels in the 7.5 ppm and 15 ppm were significantly (Tukey test, P<0.05) higher from that of control birds, and selenium levels were also significantly higher in the 15 ppm group compared to the 7.5 ppm group (Fig. 4.1).

4.3.2 Stress Response

No significant difference among treatment groups at time 0 ($F_{2,12}$=1.51, P=0.26), 15 minutes ($F_{2,12}$=0.50, P=0.62), 30 minutes ($F_{2,12}$=0.46, P=0.64) or 60 minutes ($F_{2,12}$=1.23, P=0.33) (Fig. 4.2) were detected. This resulted in no differences in the overall stress response among treatment groups (Wilks' Lambda=0.36, $F_{8,10}$=1.50, P=0.22). When blood selenium concentrations were used as a covariate with stress response, there was still no significant relationship between selenium and corticosterone concentrations (Wilks' Lambda=0.66, $F_{4,6}$=1.29, P=0.34).

4.3.3 Behavior

No differences in distance maintained between treated males and either of the resident pair birds were detected (Wilks' Lambda=0.188, $F_{16,8}$=0.65, P=0.78) (Table 4.1). There was no difference in maintenance behaviors (Wilks' Lambda=0.58, $F_{8,16}$=0.63, P=0.74) (Table 4.2) or agonistic interactions (Wilks' Lambda=0.38, $F_{10,14}$=0.86, P=0.58) (Table 4.3) observed among treatment groups. Analysis indicated no alteration of combined behavior patterns (all behaviors combined for analysis) among groups receiving different levels of selenium (Wilks' Lambda=0.046, $F_{22,2}$=0.032, P=0.93).

4.4 Discussion

4.4.1 Selenium and Stress Response

Previous studies have indicated a negative correlation between selenium and corticosterone (Freeman and Sangalang, 1977; Wayland et al., 2002). This relationship was also observed in wild scaup (chapter 2) but was influenced by interactions with mercury and body condition. Despite treatment groups in this study having different levels of blood selenium, no effect on corticosterone or stress response was detected. This lack of effect in captive individuals could be related to several factors individually or in combination. Small sample size in treatment groups may have prevented detection of any selenium-related suppression of corticosterone. The suppression of corticosterone has previously only been observed in initial blood samples representing baseline corticosterone levels (Wayland et al., 2002). Mean corticosterone levels from the first blood sample in captive individuals (5.5 ng/ml) were much lower than mean levels (37.7 ng/ml)

observed in wild birds (Chapter 2). Differences in corticosterone concentrations could be associated with a greater amount of time taken to remove birds from traps and collect samples in the field resulting in higher levels of corticosterone. In addition, wild strain birds in captivity are constantly exposed to various stressors such as human disturbance, confinement in a small rooms or competition for access to water and food. This could cause excessive adrenal activity and subsequent exhaustion of corticosterone, resulting in reduced baseline levels. Other studies have found that the stress response in captive birds may not reflect studies of wild, free-living birds (Silverin, 1998), although, typically, captive birds have higher corticosterone concentrations than free-living birds of the same species (Marra et al., 1995; Romero and Remage-Healy, 2001). Alternatively, captive rearing may have acclimated Scaup to human presence or disturbance. This could reduce the perceived threat posed by researchers upon entering enclosures limiting the corticosterone levels in captive birds. In previous studies, hand-reared birds have been shown to have lower corticosterone response to handling stress than parent-reared birds (Adams et al., 2005) and could indicate a degree of habituation to human disturbance in birds. Low baseline levels of corticosterone in captive Scaup, could have obscured or eliminated any suppressive effects of selenium on corticosterone levels and account for the lack of difference between treatment groups.

4.4.2 Behavior

Although no differences in behavior patterns were detected among

treatment groups in this study, other studies have found relationships between corticosterone concentrations and behavior. Elevated corticosterone concentrations in response to stressors act to suppress unnecessary behaviors and redirect them toward those which improve survival, such as flocking, nest abandonment or altered foraging patterns (Silverin, 1998; Perfito et al., 2002). Elevated corticosterone can also inhibit sexual behavior or stimulate escape response (Sapolsky et al., 2000). Corticosterone mobilizes energy reserves needed in resolving conflicts with other males, defending territories or establishing pair bonds (Sorenson et al., 1997) and, therefore, could influence the behavioral response to agonistic encounters with conspecifics.

The lack of any changes in behavior patterns could be explained by the absence of any selenium-induced changes in corticosterone levels. Another possible explanation could be associated with conditions within the Animal Care Unit. Light conditions within the Animal Care Unit are changed to mimic natural light conditions throughout the year. This study occurred from September until late October and as a result, photoperiod was decreasing. This indicates that aggression associated with territoriality or mate acquisition was likely low as this study did not take place within the breeding season. This may have limited aggressive or competitive interactions between introduced males and resident pair, further obscuring any possible behavioral alteration by selenium.

4.5 Conclusions

Captive studies examining the effects of contaminants on physiological

processes have the potential to clarify and simplify interpretation of results. This is especially true for general responses such as stress response which can be influenced by a wide variety of variables. Corticosterone levels can be influenced by environmental conditions, encounters with conspecifics, presence of predators, contaminants and other variables. Further complicating analysis is the potential for the interaction between variables such contaminants, body condition or pair status (Chapter 2), which can modify their effects on corticosterone levels and stress response. By using captive individuals in a controlled environment, changes in weather conditions, differences in life history or interaction of multiple contaminants could be eliminated providing clear results regarding effects of selenium on corticosterone levels.

However, using captive individuals introduces other variables which could affect results. Regular exposure to human disturbance, limited space, and unnatural diet could all potentially influence stress response and complicate interpretation. These influences limit ability to extrapolate results to wild populations. A negative relationship between corticosterone and selenium was observed in wild populations of Lesser Scaup when considered in interactions with mercury and body condition (Chapter 2). Although no effects of selenium were observed on corticosterone levels in this captive study, hormone levels do not appear to mimic responses of wild individuals. This limits the ability to draw meaningful conclusions regarding the reason for the absence of any negative correlation between corticosterone and selenium and highlights the limitations of captive studies. An additional

complication in this study was limited captive population from which to draw a sample. This low sample size was exacerbated by the loss of several birds prior to sampling blood selenium levels, corticosterone and behavior. The lack of correlation observed between selenium levels and corticosterone must therefore be interpreted cautiously.

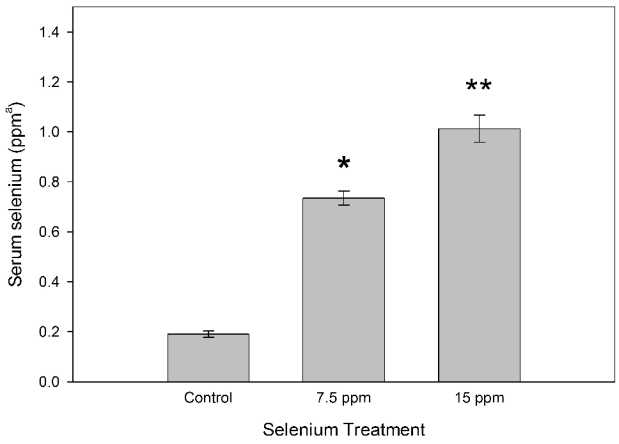

Figure 4.1 Mean (± sd) blood selenium concentrations (ppm) for male Lesser Scaup fed different levels of selenium (control, 7.5 ppm and 15 ppm). ∗ - indicate blood selenium levels which differ significantly (ANOVA, Tukey Test, $P<0.05$) from birds fed control diet. ∗∗ - indicate blood selenium levels which differ significantly (ANOVA, Tukey Test, $P<0.05$) from birds in both control and 7.5 ppm treatment groups.

[A] parts per million dry weight

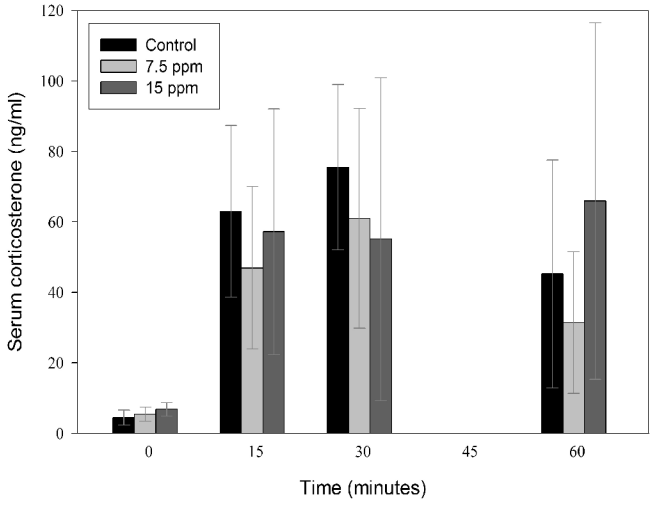

Figure 4.2 Mean (± sd) serum corticosterone (ng/ml) concentrations in blood samples collected at 0, 15, 30 and 60 minutes from male Lesser Scaup fed different levels of selenium (control group, 7.5 ppm and 15 ppm). No significant (Wilks' Lambda=0.360, $F_{8,18}=1.502$, $P=0.225$) difference in stress response was detected among treatment groups.

Table 4.1 Mean (± sd) distance of males from each treatment group from female and male of resident birds in observation room. Distance was estimated based on the number of duck lengths (female length) separating individuals. Distances were recorded each minutes during the 30 minute observation period and values indicate mean number of instances at each distance for different treatment groups. No differences (Wilks' Lambda=0.188, $F_{16,8}$=0.65, P=0.78) in distance maintained from pair were observed.

	Distance from female				**Distance from male**			
Selenium treatment	1 duck length	2 duck lengths	3 duck lengths	>3 duck lengths	1 duck lengths	2 duck lengths	3 duck lengths	>3 duck lengths
Control diet	18.2 ± 6.3	11.4 ± 6.7	0.0	0.0	22.4 ± 2.3	7.2 ± 2.2	0.0	0.0
7.5 ppm diet	10.5 ± 7.6	14.0 ± 6.8	4.2 ± 6.9	1.5 ± 3.2	17.0 ± 8.1	8.7 ± 3.0	3.5 ± 6.7	0.8 ± 1.6
15 ppm diet	9.3 ± 7.5	18.3 ± 4.5	2.0 ± 3.5	0.7 ± 0.6	18.0 ± 4.6	10.0 ± 2.6	1.7 ± 2.1	0.0

Table 4.2 Mean (± sd) number of maintenance behaviors observed in males from each treatment group. All instances of behaviors were recorded throughout the 30 minute observation period and values indicate mean number of each behavior observed for different treatment groups. No difference (Wilks' Lambda=0.579, $F_{8,16}$=0.63, P=0.74) in mean number of behaviors was observed among groups.

Selenium treatment	headshake [a]	preen[b]	dive [c]	forage/drink[d]
Control diet	17.6 ± 12.1	4.8 ± 4.9	0.4 ± 0.9	2.8 ± 2.2
7.5 ppm diet	12.2 ± 9.9	4.0 ± 4.7	0.7 ± 1.6	1.2 ± 1.5
15 ppm diet	20 ± 7.5	9.7 ± 10.0	2.7 ± 4.6	3.7 ± 1.5

[a] shaking of the head not associated with other behaviors (i.e. diving, preening)
[b] bathing and grooming of feathers
[c] any movement while submerged in the pool
[d] includes eating at food dish and drinking or foraging in the pool

Table 4.3 Mean (± sd) number of agonistic interactions observed in males from each treatment group. All instances of behaviors were recorded throughout the 30 minute observation period and values indicate mean number of each behavior observed for different treatment groups. No difference (Wilks' Lambda=0.383, $F_{10,14}$=0.86, P=0.58) in mean number of behaviors was observed among groups.

Selenium treatment	pecked male	pecked by male	pecked female	pecked by female	chased male	chased by male	chased female	chased by female
Control diet	0.4 ± 0.5	4.4 ± 5.6	0	5.2 ± 5.2	0	0.8 ± 1.8	0	2.6 ± 3.6
7.5 ppm diet	0	4.7 ± 5.2	0	4.2 ± 5.8	0	0.7 ± 1.2	0	4.2 ± 6.6
15 ppm diet	0.7 ± 0.6	2.3 ± 2.5	0	0	0	0	0	1.7 ± 2.9

5.0 General Discussion

Contaminants have been cited as possible contributors to a decrease in Scaup productivity and thus, to a decline in the mid-continental Scaup population. Of contaminants measured, selenium has been repeatedly detected at levels which could potentially affect reproductive success in females (Custer et al., 2000; Custer and Custer, 2002; Petrie 2004). Concentrations of approximately 10 µg/g dry weight in liver of female Mallards have been correlated with birth defects in developing embryos (Heinz,1996). Although concerns about the potential reproductive effects of selenium tend to focus on females, selenium concentrations in female tissue can decline as selenium is deposited in eggs (Heinz, 1996). Tissue selenium concentrations in female scaup on breeding grounds, therefore, may not accurately represent the risk of reproductive effects. Since male tissue selenium concentrations do not decline due to deposition in eggs, male concentrations may more accurately represent population selenium on the breeding grounds. If females have similar selenium concentrations to males in this study, it is possible some females may arrive on breeding grounds in the Northwest Territories with levels

which could affect reproduction.

Contaminant concentrations in all individuals in this study were below levels considered potentially lethal (Furness, 1996; Heinz, 1996; Thompson, 1996). Contaminants were associated with sublethal changes to physiology including modification of hormone secretions. As in other studies of ducks (Wayland et al., 2002), cadmium and selenium influenced corticosterone levels. In my study, however, the influence of contaminants on hormone secretions were modified by interactions between several variables including other contaminants, body condition and size and pair status. Results appear to support other studies which have observed a suppression of corticosterone by selenium (Freeman and Sangalang, 1977; Wayland, 2002). This suppression was observed in birds with high cadmium and good body condition but not in birds with poor body condition. Cadmium can stimulate the hypothalamic-pituitary-adrenal axis resulting in elevation of corticosterone (Hidalgo and Armario, 1987; Wayland, 2002). Birds in good condition may be producing higher levels of metallothionein, which binds and limits bioavailability of cadmium. This could reduce elevation of corticosterone by cadmium and allow the suppression of corticosterone by selenium to be visible. Birds in poor condition may not produce adequate metallothionein levels, resulting in stimulation of corticosterone levels by cadmium which could obscure any suppressive effects of selenium.

The negative correlation between selenium and corticosterone was also observed in birds with low cadmium and low mercury. In birds with high mercury,

however, no relationship was observed between selenium and corticosterone. This interaction between selenium and mercury in my sample appears to support other research which has found evidence of selenium and mercury interactions, often reducing expression of toxic effects of these elements (Cuvin-Aralar and Furness, 1991). High mercury levels, may result in formation of selenium-mercury complexes, preventing suppression of corticosterone by selenium. Low mercury levels, however, may limit formation of these complexes allowing free selenium to suppress corticosterone levels, resulting in the observed negative relationship. An interaction of mercury levels and body size also influenced corticosterone levels in birds with high cadmium. Small birds showed a negative relationship with corticosterone while no relationship was observed in large birds. The mechanism of this interaction between cadmium, mercury concentrations and body size requires further research.

Hormone levels were also altered by interactions of pair status and contaminants in this study. In birds with high cadmium levels, no difference in corticosterone levels were observed between paired and unpaired birds. In birds with a low cadmium status, however, unpaired birds had higher corticosterone levels than paired birds. Though the reason for this interaction between cadmium and pair status is not certain, high cadmium levels could be elevating corticosterone obscuring differences between individuals of different pair status. Higher corticosterone levels in unpaired individuals could mean that they are experiencing a higher degree of stress during the breeding season. These results are consistent

with studies of geese during mating season, where unpaired or subordinate males had higher fecal corticosterone compared to paired males (Kotrschal et al., 1998). It was proposed that this higher degree of stress in subordinate males could be associated with unsuccessful attempts to acquire mates or because they are more frequent targets for aggression from dominant males (Kotrschal et al., 1998).

In the present study, thyroxine was also influenced by an interaction of contaminants, in this case mercury, and pair status. Paired males had a positive relationship between mercury and thyroxine while no relationship was observed in unpaired birds. Exposure to mercury has been associated with elevation of thyroxine levels (Barregard et al., 1994; Bleau et al., 1996). It has been suggested that mercury in the liver could inhibit the action of deiodinase enzymes thereby limiting the conversion of thyroxine to triiodothyronine (Sin et al., 1990). This could result in higher thyroxine levels in individuals with higher liver mercury levels. The highest mercury concentrations in male scaup were seen in paired individuals and this could account for the positive correlation with thyroxine levels. Prolonged exposure to stress and elevated corticosterone have been shown to decrease thyroid hormone concentrations (Kuhn et al. 1998; Williamson and Davidson, 1987). Unpaired males with low cadmium had higher corticosterone levels which could indicate they experience more prolonged or higher degree of stress during the mating season. This elevation of corticosterone could result in suppression of thyroxine secretions, thereby limiting the effects of mercury and obscuring any positive correlation with thyroxine.

Interactions of contaminants, body condition, body size and pair status did not affect testosterone levels or testes mass in my sample. Testosterone levels relate to aggression, courtship behaviors and secondary sexual characteristics. Testicular mass can influence testosterone levels with larger testes secreting higher quantities of testosterone. Testosterone levels, however, are dynamic and once mate selection has occurred, levels can decline (Sorenson et al., 1997). It would be more suitable, therefore, to measure effects of contaminants prior to formation of stable pair bonds, during courtship and early mating when peak testosterone levels occur. This would clarify whether contaminants can influence reproductive readiness or ability to attract a mate in Lesser Scaup males by altering reproductive physiology or hormones. It would likely also be more appropriate to measure the influences of hormonal and physiological variables on pair status prior to, or during pair bond formation. In the present study, only body condition and body size appeared to affect pair status. Testosterone, testes mass, corticosterone and testicular asymmetry were not related to pair status. Assessment of these variable at time periods more relevant to the measured endpoints (i.e. during competition for mates) could provide a more complete characterization of those variables most important for mate acquisition in Lesser Scaup.

Testicular structure, specifically seminiferous tubule diameter, was associated with selenium levels. As in other studies (Green and Albers, 1997; Zhang et al., 2004), selenium concentrations in the present study showed a negative relationship with tubule diameter. Although the mechanism of the relationship is

poorly understood, it could be associated with altered testosterone synthesis or concentrations of selenium containing proteins in gonadal tissue (Behne et al., 1996). Testosterone concentrations were not associated with selenium in my sample. As previously discussed, selenium may have only affected testosterone levels earlier in the season prior to pair bond formation. Alternatively, selenium related changes in testosterone may have only been observed during periods of peak selenium exposure while staging on the Great Lakes. Changes in testicular structure may provide evidence of more long term changes in Scaup physiology associated with elevated selenium, and could therefore be a better indicator of selenium toxicity than measurement of dynamic endpoints, such as hormone levels. Results from the field portion of this study emphasize the complex nature of biological systems and emphasize the importance of considering interactions of multiple variables when attempting to characterize the effects of contaminants.

In captive male Lesser Scaup, selenium did not suppress corticosterone secretions or alter behavior patterns. Captive studies such as this one, provide an opportunity to examine endpoints in a controlled environment. This has the potential to limit the influence of factors such as environmental conditions which may affect stress related variables and complicate interpretation of results. Using captive individuals, however, introduces other factors which could influence results. Regular exposure to human disturbance, limited space, and unnatural diet could all potentially alter stress response, corticosterone levels and behavior patterns. These influences limit ability to extrapolate results of captive stress studies to wild populations and, therefore, negative results must be interpreted cautiously.

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